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Mechanism of Zinc Sulphate Prophylaxis in Experimental Poliomyelitis.

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We have previously reported[1] that the application of 1% zinc sulphate to the olfactory mucosa of monkeys affords protection against poliomyelitis virus inoculated intranasally one or more months later. The basis of this protection is not yet understood.

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[1] Schultz, E. S., and Gebhardt, L. P., *J. Am. Med. Assn.*, 1937, **108**, 2182.

Nasal mucous membranes removed a week or more after treatment generally present a normal appearance. Recently, however, we have studied membranes removed several hours to several days after treatment with varying concentrations of zinc sulphate solution.

With a little experience it is possible to remove olfactory mucosa, olfactory nerve filaments and olfactory bulbs, of both sides, all in one piece. These have been embedded and sectioned tangentially. Such sections afford the orientation necessary for a satisfactory study of the olfactory mucosa and related structures and within limits allow comparison of treated and untreated sides.

Our observations show that by the end of the first day, there may be a marked acute inflammation, associated with some desquamation and necrosis of epithelial cells. Following treatment with 1.5% zinc sulphate solution, this change may be so marked that much of the olfactory mucosa loses its epithelium. The inflammatory reaction tends to subside quickly and is followed promptly by regeneration. By the end of the 2nd day denuded areas may be again covered with an epithelium consisting of flat or cuboid cells with many cells showing mitosis. By the end of the 3rd, 4th or 5th day the epithelial surface again appears normal, except for some disorder in the arrangement of the cells and for a few scattered collections of PMN cells.

It is generally held that destroyed nerve cells are not restored. We are, however, not aware of any observations proving that this holds also for olfactory cells. Should the protection induced in monkeys by zinc sulphate be the result of a destruction of olfactory cells and not the result merely of an injury or chemical change in the dendrites of the olfactory neurons, it would then seem to follow that these structures are in reality regenerated in some way. This statement is justified in part on the ground that the majority of treated resistant monkeys become susceptible again in 2 to 4 months. Either the protection is not associated with a complete destruction of olfactory cells or certain cells in the olfactory area are differentiated into cells capable of forming axons and dendrites and of eventually restoring function. Such a provision does not seem entirely unlikely in a structure which is probably not altogether free of the risk of naturally induced injuries.

During the past summer, zinc sulphate solution was applied to the olfactory mucosa of several thousand persons. A thorough application is said to always produce an anosmia. In children, this disappears within 2 weeks. In adults, however, the sense of smell may not return for several months. This, together with the fact that adults frequently suffer severe pain immediately following treatment, suggests that these differences may be explained on an anatomic basis. In adults, the superior meatus is narrower than in children,[2] it may indeed be narrow and deep enough to contribute materially to capillary attraction. This would result in the solution being held in contact with the mucosa for a longer period of time and thus add not only to the discomfort, but also to the duration of chemical action on the cells. The question of the possible risk of inducing permanent anosmia by such more prolonged and uncontrolled drug action will not be discussed here; nevertheless, it is of considerable theoretical interest that a few cases in adults are known to us in whom the sense of smell has been completely, or nearly completely regained after an anosmia lasting 2 to 4 months. This is in harmony with our observation that treated monkeys generally become susceptible again to intranasal inoculation of virus within 2 to 4 months after treatment. This raises the question, what is the mechanism underlying these late restorations of function in man and of susceptibility to infection in monkeys?

9952

Listerella Monocytogenes: A Cause of Meningo-Encephalitis in Man.

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In a preliminary note[1] we reported the isolation from a human case of meningo-encephalitis of a new organism which we identified with the genus Corynebacterium. We wish at this tme to report additional observations and to correct the classification of the organism.

The clinical history of the case is described in the preliminary note and has been more fully presented by Marcellus, Crouch and

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[2] Shahinian, L., Bacher, J. A., McNaught, R. C., and Newell, R. R., *J. Am. Med. Assn.*, 1938, **110**, 1254.

[1] Schultz, E. W., Terry, M. C., Brice, A. T., and Gebhardt, L. P., PROC. SOC. EXP. BIOL. AND MED., 1934, **31**, 1021.

Terry.[2] A noteworthy fact is that the organism was cultured from the spinal fluid of this patient 12 times over a period of nearly 4 months, though the period of acute illness lasted less than 2 weeks.

A month after our report, Burn[3] in Connecticut reported the isolation of a similar organism from 3 fatal cases of meningo-encephalitis in infants. Later,[4] he reported a fourth fatal case, this time in an adult, and identified his strains with the genus Listerella. In this paper he refers also to a case reported to him by Dr. W. Allen of Hartford, Connecticut. From a fatal case of meningitis in a human adult in Scotland, Gibson[5] isolated an organism which he thought belonged to the genus Corynebacterium. Morphologically and culturally the organism resembled our strain. In Norway, Tesdal[6] described a case of fatal meningitis in man "caused by a Corynebacterium." His description of the organism, however, suggests a Listerella. More recently Carey[7] in Massachusetts has isolated a Listerella from a case of acute cerebrospinal meningitis in a boy. This patient recovered. These seem to comprise all the cases of human infection known to have been caused by Listerella.

Since publishing our preliminary note[1] our organism has been definitely identified as *Listerella monocytogenes,* originally described by Murray, Webb and Swann[8] and isolated by them from a spontaneous disease in rabbits characterized by monocytosis. Similar organisms have also been recovered from a variety of diseases in animals, including a plague-like disease in gerbille in South Africa, meningo-encephalitis in sheep and in cattle, and a disease in chickens associated with massive myocardial necrosis (for a review see Seastone[9] and Jungherr[10]).

The morphology of the organism is described in our preliminary report. Grown on most media it is a short plump (0.5x1-2 μ) gram-positive rod. On certain media it shows marked variation in size and form.[1] We wish to emphasize a morphological feature easily overlooked, namely, the presence of flagella and of motility.

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[2] Marcellus, M. B., Crouch, E. L., and Terry, M. C., *Northwest. Med.*, 1936, **35**, 50.

[3] Burn, C. G., (*a*) PROC. SOC. EXP. BIOL. AND MED., 1934, **31**, 1095; (*b*) *Am. J. Path.*, 1935, **11**, 856.

[4] Burn, C. G., *J. Bact.*, 1935, **30**, 573; *Am. J. Path.*, 1936, **12**, 341.

[5] Gibson, H. J., *J. Path. and Bact.*, 1935, **41**, 239.

[6] Tesdal, M., *Acta Med. Scand.*, 1934, **83**, 351.

[7] Carey, B. W., *J. Pediatr.*, 1936, **8**, 626.

[8] Murray, E. G. D., Webb, R. A., and Swann, M. B. R., *J. Path. and Bact.*, 1926, **29**, 407.

[9] Seastone, C. V., *J. Exp. Med.*, 1935, **62**, 203.

[10] Jungherr, E., *J. Am. Vet. Med. Assn.*, 1937, **91**, 73.

We originally classified our strain as a Corynebacterium solely because non-motile, gram-positive, non-acid fast, non-spore bearing, aerobic, rod-like bacteria are usually placed in this genus. Both Gibson[5] and Tesdal[6] classified their strains as Corynebacteria. Burn[3a] reported his strains as "very sluggishly motile"; later,[3b] he stated that he had been unable to demonstrate either motility or flagella and in a final report,[4] that "there was evidence of true motility." The observations of Seastone[8] have been particularly helpful on this point, both with regard to the type motility exhibited by these organisms and the limited cultural conditions under which it is seen. As he has pointed out, it is seen best in 4-hour dextrose broth cultures. He found the type of motility to be rather unusual: "In any one field in a hanging drop, a few individuals will be seen actively moving in a tumbling or spiral manner. These may cease and others commence, often with a preliminary violent spinning." With a modified Leifson flagella strain he demonstrated flagella. We have been able to confirm these observations.

We have compared the cultural and biochemical properties of our strain with 11 other strains of Listerella. These include a strain from Murray, *et al.*, (58.xxiii);[8] the Pirie strain,[11] 4 human strains isolated by Burn[4] and 4 animal strains (Fowl 5407, D-82N. Calf 1864, Cow B-1946) from Seastone[9] and the human strain isolated by Gibson.[5] All have presented essentially the same characters of growth and biochemical properties previously described[1] for our strain. All strains form colonies which are circular, sharply circumscribed, finely granular, domes or cones. At the summits of some there may be a characteristic umbilication or "mortar splash." Viewed by soft indirect light at a magnification of about 30× the colonies are steel gray, silvery, lusterous arcs or cones, which readily reflect the grating of windows or other objects focused on them. On blood agar the colonies are surrounded with a zone of partial (almost complete) hemolysis. On superficial examination the growth on blood agar plates resembles somewhat that of streptococci. All strains ferment xylose, dextrose, levulose, sucrose, maltose, lactose, soluble starch (Pfanstiehl), rhamnose, dextrin and glycerol. Sucrose, lactose and glycerol are fermented late. Galactose is fermented feebly or not at all. A trace of acid was formed by 3 strains, including our strains, the Murray strain and a strain (D-82N) obtained from Seastone; the remainder did not attack this carbohydrate. None attacked arabinose, inulin, raffinose, erythritol, mannitol, dulcitol, adonitol, or inositol. All reduced litmus, but none reduced nitrates. None digested coagulated blood and none liquefied gelatin. Lead acetate agar was not blackened and no indol was formed.

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[11] **Webb, R. A., and Barber, M., *J. Path. and Bact.*, 1937, 45, 523.**

Antisera prepared against our strain agglutinate the Murray, the Pirie and the Gibson strains in about the same dilutions of serum (1:5120); while the same antisera agglutinate the Burn and the Seastone strains only in low dilutions (1:80 to 1:160). Sera produced against the Burns and Seastone strains agglutinate these strains in high dilutions, but the Murray, Pirie, Schultz and Gibson strains only in low dilutions or not at all. These observations harmonize with previous reports (Seastone,[9] Webb and Barber[11]) and confirm the fact that the New England strains, while morphologically, culturally and biochemically similar to our strain and to the British strains, differ serologically.

No Listerella infections in animals have thus far been reported on the Pacific Coast, and the source of the infection in our case has remained a mystery.

While this report was waiting to be written up, an account of a comparative study of some of these Listerella strains by Webb and Barber[11] appeared. This note confirms and supplements their observations.

9953 P

Suppression of *Pars Intermedia* of Pituitary Body in *Hyla regilla* by Operations Upon the Gastrula.

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The dual character of the pituitary body and the constant association of its neural and epithelial components throughout the vertebrate sub-phylum, have raised the question of whether or not association of the components is necessary for the development of the gland. Blount (1930), Etkin (1935) and Atwell (1935, 1936) have contributed toward the solution of the problem by means of grafting experiments, performed upon tail-bud stages of early larvae of various amphibian species. This paper is a preliminary account of results obtained by means of a different attack upon the problem.

Smith (1916), Allen (1916) and Atwell (1919) have shown that hypophysectomy in the early anuran larva produces a pigmentary condition which is characterized by aggregation of the melanin granules in the melanophores, dispersion of the pigment granules of the Xantholeucophores and a general reduction of free melanin in the epidermis. Such tadpoles are silvery-white in appearance and have been described as "albino". This pigmentary condition has been shown to prevail in the absence of the *pars intermedia* of the pituitary body.

It has been possible to produce the *pars intermedia* deficiency syndrome in tadpoles of *Hyla regilla* in 2 different ways, by microsurgical operations made as early as the gastrula stage.

In the first operation a small, rectangular piece of tissue, including the 3 germ layers, is removed from the presumptive medullary region, at a position approximately 95 degrees forward from the dorsal lip of the closing blastopore. This piece of tissue, the width of which is about one-fifth that of the gastrula, is turned end for end and reimplanted. Thus the lateralities of the tissue are reversed, but more important in this connection is the fact that originally anteriorly situated materials are shifted to a more posterior position and *vice versa.* Tadpoles derived from these gastrulæ are "albino".

Microscopic sections of these experimental larvæ reveal that the infundibular region of the brain has been shifted to a position posterior to its normal location, and forms from the floor of the myelencephalon instead of from the diencephalon. In the course of development, the *pars buccalis* arrives at its definitive location, at the anterior end of the notochord, but fails to reach the infundibulum. The *pars buccalis* remains as a small, darkly staining, comparatively undifferentiated mass of tissue. This microscopic picture has been found in every one of 14 serially sectioned larvæ which had been picked at random from a total of 67 experimental "albinoes".

A second series of experiments, which also causes the *pars intermedia* deficiency syndrome involves the transplantation of the gastrular anlage of the *pars buccalis.* By means of vital staining technic this anlage has been located at a position in the midline of the embryo, approximately 160 degrees forward from the dorsal lip of the blastopore. The presumptive *pars buccalis,* together with its substrate, is excised and interchanged with a small square of tissue taken from the dorsal side of the gastrula a short distance anterior to the dorsal lip of the blastopore. This latter piece of tissue includes the 3 germ layers.

Microscopic sections of tadpoles derived from these gastrulæ show the *pars buccalis* to be entirely lacking in its normal location beneath the infundibulum. It is present, however, as a distant and characteristic body of cells closely applied to the floor of the myelencephalon, where it appears to have induced an hyperplasia of the adjacent tissue of the hindbrain. At this position, a section of the notochord is missing, but this segment of the notochord is to be found just anterior to the infundibular recess, beneath the forebrain. It is to be remembered that the rudiment of this piece of chorda was placed in the position normally occupied by that of the *pars buccalis* in the gastrula stage. Since at that time chordal material was irreversibly determined, its occurrence in this anterior situation was to have been expected.

By the first experiment it is shown that prevention of contact between the *pars buccalis* and nervous tissue, by microsurgical intervention at an early embryonic stage, is equivalent to removal of the *pars intermedia* at a later stage, since the development of the *pars intermedia* is suppressed. The second experiment establishes the location in the late gastrula of the presumptive *pars buccalis,* and indicates that the neural tissue necessary to cause the differentiation of the *pars intermedia* is more or less specific. Although it cannot be said at this time that the infundibular region of the brain possesses the inductive power exclusively, it is believed that tissue with this power must be similar to that of the infundibulum. It is suggested that the ependymal cells may have this capacity.

9954

Adrenal Cortical Hormone (Cortin) in Blood and Urine of Patients with Cushing's Disease.*

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Evidence has been accumulating which tends to support the hy-

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pothesis that the symptoms of Cushing's disease are due to a hyperfunction of the adrenal cortex.[1] If this is true, one might expect to find an increased amount of adrenal cortical hormone (ACH) in the blood of these patients. We have previously shown that the sera of patients with Cushing's disease, when injected into adrenalectomized rats, prolong the survival period of these animals beyond that of untreated controls.[2] The present communication reports the fact that extracts of the patients' blood and urine prepared by a method used for extracting ACH from adrenal tissue will also prolong the lives of adrenalectomized rats. This would seem to add further support to the hypothesis that Cushing's disease is due to an overproduction of the adrenal cortical hormone.

Grollman and Firor[3] have demonstrated that ACH can be obtained from the urine of normal adults in very small amounts. Utilizing as their standard the growth and the prolongation of life in adrenalectomized rats, they found that one liter of urine contains an amount of hormone (ACH) corresponding to 0.5 g of glandular tissue.

Extracts were made of the blood of 3 patients with unmistakable Cushing's disease. In one case the urine was also extracted. In a fourth patient, a girl of 5 with an adrenogenital syndrome, the urine was tested in the same way. The extraction was carried out according to the method of Grollman and Firor, using benzene. The residue from 100 cc of blood was taken up in 10 cc of olive oil. The residue from a 24-hour urine specimen was also taken up in 10 cc olive oil. The extract was injected subcutaneously in doses of 0.1 cc daily into male rats adrenalectomized at one month of age. Only those rats which had gained at least 5 g in weight by the fifth postoperative day were used for the assay. It was found that rats which had not gained this amount were likely to die in 2 or 3 days, probably as the result of the operation, even when significant amounts of ACH (Upjohn's preparation) were given. The assay injections were started on the fifth day.

The test animals were fed a stock diet consisting of:

Whole wheat	67.5
Casein	15.0
Whole milk powder	10.0
NaCl	1.0
$CaCO_3$	1.5
Milk fat	5.0

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[1] McQuarrie, I., Johnson, R. M., and Ziegler, M. R., *Endocrinol.*, 1937, **21**, 762.

[2] Anderson, E. M., and Haymaker, W., *Science*, 1937, **86**, 545.

[3] Grollman, A., and Firor, W. M., PROC. SOC. EXP. BIOL. AND MED., 1933, **30**, 669.

TABLE I.
Survival of Adrenalectomized Rats Treated with Extracts of Blood and Urine of Patients with Cushing's Disease.

Daily	Daily Dose Equivalents	No. of Test Animals	Age at Time of Adrenalectomy, days	Days Survival After Adrenalectomy	
				Range	Median
Control Groups					
1. No injections		28	29†	6-15	8.1
2. Injected with olive oil	.2 cc	11	29	7-14	9.1
3. ” ” ACH (Upjohn)	.1 cc aq. soln. = 4 g fresh gland	4	28	10-14	11.8
4. ” ” ” ”	.2 ” ” ” = 8 ” ” ”	2	29	10-17	13.3
5. ” ” ” ”	.2 ” ” ” = 8 ” ” ”	12	29	23-33*	26.6*
Experimental Groups					
1. Extract of blood, Patient A (Cushing's Disease)	.1 oil soln. = 1 cc blood	10	27†	7-30	14.5
2. Extract of blood, Patient B (Cushing's Disease)	.1 ” ” = 1 ” ”	2	26†	16	16.0
3. Extract of urine, Patient B	.1 ” ” = .01 of total 24-hr urine	23	27	8-32	16.1
4. Extract of blood, Patient C (Cushing's Disease)	.1 ” ” = 1 cc blood	5	27	9-20	14.2
5. Extract of urine, Patient D (Adrenogenital syndrome)	.1 ” ” = .01 of total 24 hr urine	8	27	7-15	10.4
6. Extract of urine, Normal Adult	.1 ” ” = .01 ” ” ” ”	7	29	6-12	8.5
7. Extract of Blood, Normal Adult	.1 ” ” = 1 cc blood	8	28	7-13	9.6

*Injections of ACH were stopped on 20th day postoperative. The average survival period after stopping the hormone was 6.6 days.
†Age differences of 2 or 3 days at this period of life influence survival periods, the older rat having a longer survival period.

On this diet the average survival period of adrenalectomized rats from our colony and of this age was 16 days. In order to shorten the survival period, a solution of 0.5% KCl was given in the drinking bottles in place of tap water. This reduced the average survival period to 8.1 days.

The results of this study are given in Table I. It is evident that the adrenalectomized rats receiving extracts of blood and urine from patients with Cushing's disease survive a longer period that the untreated controls. An attempt has been made to estimate roughly the potency of these extracts by comparison with known amounts of ACH (Upjohn). It would appear that 1 cc of blood from a patient with Cushing's disease contains an amount of ACH equivalent to slightly more than the hormone content of 4 g of fresh adrenal glands. A 24-hour urine specimen may contain an amount equivalent to 400 g of tissue. The urine of the patient with the adrenogenital syndrome showed no significant amount of ACH; however the patient did excrete androgenic hormone as follows per 24 hours: 5 IU of free androgenic hormone and 33 IU of hydrolyzable androgenic hormone.

The question might be raised whether the presence of oestrin or of prolan, which may both be present in the urine, might prolong the life of adrenalectomized rats, but Grollman and Firor found that these substances had no effect upon prolonging the lives of these animals. This is being checked further in a special study.

Conclusion. Patients with Cushing's disease have in their blood an excess amount of a substance which resembles adrenal cortical hormone in its ability to prolong the life of the adrenalectomized rat. Furthermore, this substance is excreted in significant amounts by the kidneys.

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Effect of Vitamin A Deficiency upon Rate of Pupil Dilation During Dark Adaptation.

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Wald has recently shown that there is a definite chemical relationship between visual purple in the retina and vitamin A. The visual processes of light and dark adaptation form a cycle, vitamin A being the breakdown product of visual purple, as well as the precursor. It is found mainly in the light adapted retina, and only a trace exists in the dark adapted one. The latter, however, contains relatively large amounts of visual purple.[1, 2]

Wald has further shown that bleached isolated retinas contain much more vitamin A than retinas from light adapted animals. He concludes from this that the visual purple system loses vitamin A and relies upon the diet for its replacement. These results form an excellent basis for the explanation of night blindness caused by vitamin A deficiency.

If the stimulation of the rods, in which the visual purple is contained, has anything to do with the pupillary reflex, the measuring of the rate of dark adaptation, determined by changes in pupil diameter, might form the basis for an objective test for the determination of night blindness.

The experimental work done thus far consists of the measuring of the rate of pupil dilation during dark adaptation, by a method previously described.[3] This, in brief, involved the constricting of the pupil by an intense stimulus light followed by the taking of a series of photographs in virtually complete darkness, by means of an infrared source of light. The rate of pupillary constriction was also determined in the latter parts of the experiments, using a method described in a later paper.[4]

Nine rabbits, from 6 to 8 weeks of age, were placed on the vitamin A-free diet, consisting of rolled oats, 60 parts; dry skimmed milk, 30 parts; and dry brewer's yeast, 10 parts. A few drops of Viosterol were administered by mouth every week.

A series of the photographic measurements was taken to determine the pupil dilation during dark adaptation on each animal before it was placed on the restricted diet. The group was then divided into 2, and the measurements were repeated every week, taking each half alternately.

The diet and measurements were kept up for 92 days, during which time weight records of each animal were obtained. There was a steady rise in weight until the 56th day, when 2 of the rabbits showed a loss in weight. By the 70th day, all the animals showed

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[1] Wald, G., *J. Gen. Physiol.*, 1935, **18**, 905.

[2] Wald, G., *J. Gen. Physiol.*, 1935, **19**, 351.

[3] Gullberg, J. E., Olmsted, J. M. D., and Wagman, I. H., *Am. J. Physiol.*, 1938, **122**, 160.

[4] Gullberg, J. E., Olmsted, J. M. D., and Wagman, I. H., PROC. SOC. EXP. BIOL. AND MED., 1938, **38**, 616.

a marked decline. The animal which showed the greatest decline died on the 64th day, and exhibited clear evidence of vitamin A deficiency. On the 81st day, 2 more animals showed marked symptoms, including clouding of the cornea and swelling of the eyelids. The animal which showed the most severe signs was fed pure carotene every day for 5 days. Definite response to the administration of carotene was evident on the 2nd day, and there was a progressive improvement until, on the 6th day, all of the superficial symptoms of the deficiency had disappeared.

An analysis was made of all the data from the pre-deficient series to the clear-cut terminal stages with the physical lesions. No significant difference in the rate of response could be detected at any point in the series. All the curves of the rate of pupil change during light and dark adaptation showed the form characteristic of the normal animals previously described.[3]

Our results are in disagreement with those of Phillips and Bohstedt who produced a syndrome in rabbits which they attributed to a deficiency of vitamin A in the diet. Among other symptoms they found that "the light reflex was sluggish and somewhat disturbed."[5]

The evidence that has been collected does not exclude the possibility of an effect existing in vitamin A deficiency which could only be revealed by a change in pupillary response to low threshold stimuli. Further work along these lines has been planned.

Laurens concludes that the pupillary reflex is primarily and more easily elicited from the cones, but that stimulation of the rods also gives it.[6] It seems to us that, with the intense, broad beam of light used as the stimulus for pupillary constriction in our experiments, the cones alone must be responsible for the pupillary reflex. If the rods have anything to do with pupillary action, the effect is probably not detectable by the method herein described.

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[5] Phillips, P. H., and Bohstedt, G., *J. Nutrition*, 1938, **15**, 309.

[6] Laurens, H., *Am. J. Physiol.*, 1923, **64**, 97.

9956

Reciprocal Action of the Constrictor and Dilator Pupillae During Light Adaptation.

J. E. GULLBERG, J. M. D. OLMSTED AND I. H. WAGMAN.
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It has been shown by infrared photographic measurements that dilation of the pupil during dark adaptation is the result of 2 mechanisms, namely, the passive elasticity of the iris tissue, and the actual contraction of the dilator pupillæ.[1] The maximum pupil diameter during normal dark adaptation is reached when the added effect of these 2 mechanisms just balances the "residual tone" of the constrictor pupillæ.

After cutting the sympathetic, the maximum pupil diameter is less than normal, and is now determined by the balance between the passive elasticity of the iris on one hand, and the "residual tone" of the constrictor pupillæ on the other.

To complete the study of reciprocal innervation of the iris musculature of the rabbit, we observed the rate of both dark and light adaptation of the eye, before and after cutting the third nerve, and before and after paralyzing the constrictor mechanism with atropine.

The photographic method used to determine the size of the pupil during light adaptation is basically the same as the method to measure the rate of dark adaptation previously described.[1] The method is so designed that even under bright light conditions the photographs are taken by illumination with an infrared source of light. There is, therefore, no need for a change in technic nor for any adjustment in the transition from virtual darkness to the condition of an intensely illuminated field if the camera can be made "blind" to the stimulus light.

The middle density infrared filter made for the Xenon F 1.5 lens was used over the lens without an appreciable increase in the exposure time of the previously described method of illumination of the eye. If the stimulus light is made relatively free of the far red and infrared components, it is possible to have the same photographic conditions for light and dark adaptation.

The stimulus light must have the qualities of high intensity combined with stability and uniformity over the considerable area which must be illuminated. For this purpose the best standard lamp is the tungsten ribbon filament, 6-volt, 108-watt bulb. This was used with an aspheric condenser in the lamp housing of Bausch and Lomb. Such a lamp throws an image of great uniformity in the form of a rectangle which has sufficient width to allow for minor head movements without change in the stimulus intensity. Care was taken to control the voltage. The far red and the infrared portions of the radiations were removed from the source by a combination filter consisting of a parallel-sided glass cell, 32 mm in depth, filled with the saturated solution of nickel ammonium sulphate, and a standard glass heat absorbing filter disc, of 2 mm thickness. This combination filter removes some of the blue violet end of the spectrum in addition to eliminating the red and infrared parts, but the residual is still very effective in pupillomotor action.

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[1] Gullberg, J. E., Olmsted, J. M. D., and Wagman, I. H., *Am. J. Physiol.*, 1938, **122**, 160.

After a series of photographs had been taken during complete dark adaptation, as previously described,[1] the stimulus light was thrown into the eye, and photographs were taken as quickly as possible, until the pupil reached a constant diameter. The action is very rapid, the average time for completion being from 5 to 10 seconds (Fig. 1). Reeves[2] obtained similar results with the normal

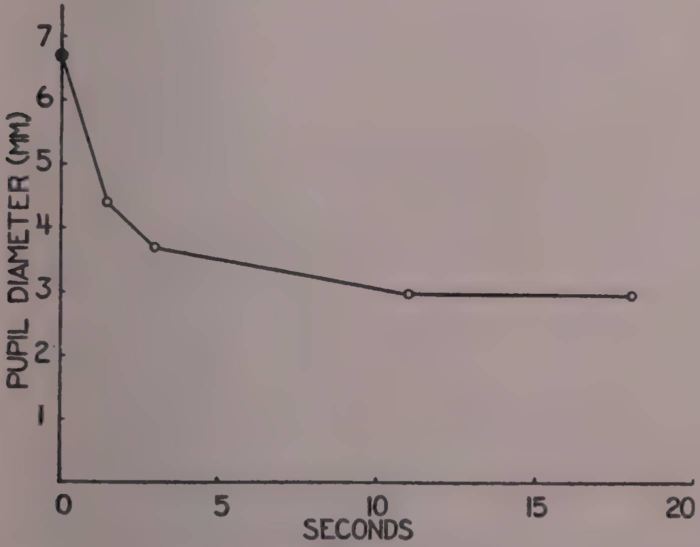

FIG. 1.

Curve showing rate of pupil constriction during light adaptation of the normal rabbit's eye.

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[2] Reeves, P., *Psych. Rev.*, 1918, **25**, 330; *J. Franklin Inst.*, 1918, **185**, 753.

human pupil. He found that it reached its minimum in less than 5 seconds, and the greater part of the constriction occurred within the first 2 seconds.

After the third nerve was cut, the pupil was permanently dilated to a greater maximum than during dark adaptation in the normal eye. No detectable response was obtained when the intense light was thrown into the eye with the third nerve cut. The same result was also observed after the direct application of 3 drops of a 2% solution of atropine sulphate to the normal eye.

It seems from our results that after cutting the third nerve, the elasticity of the iris plus the active contraction of the dilator pupillæ can now have its full effect. There is no longer any "residual tone" of the constrictor to overcome since this muscle is completely relaxed. The pupil, under these conditions, therefore, reaches the maximum diameter of which it is capable, and remains so during dark adaptation and during light adaptation as well.

As there is reciprocal action on the part of the 2 muscles of the iris, an intense light in the eye should cause a relaxation of the dilator even when the third nerve is cut or paralyzed. Such may be the case, but any constriction of the pupil that might take place under these circumstances is not detectable by the photographic method used, presumably because the range of movement of the feeble dilator muscle fibers is exceedingly slight when near or at their shortest length.

It has been found that when both the sympathetic and third nerves are cut, the iris assumes an immobile position in which the diameter of the pupil is only ¾ of the maximum which is reached when the third nerve alone is cut.[3, 4, 5] "Residual tone" has had to be postulated for the constrictor muscle in order to account for the increase in pupil diameter when atropine is instilled into the dark adapted eye after the sympathetic alone is cut. So here, with the third nerve cut, "residual tone" must be postulated for the dilator muscle in order to account for the greater diameter of the pupil when the sympathetic is intact than when it is cut. Again, as in the case of the constrictor, this "residual tone" is independent of the working of its antagonist in its reciprocal action.

Our experiments show that, while dilation of the pupil can be brought about by relaxation of the sphincter alone, relaxation of the dilator plays no appreciable part in constriction. This agrees with

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[3] Parsons, J. H., *J. Physiol.*, 1901, **26**, 366.

[4] Parsons, J. H., *Roy. London Ophth. Hosp. Rep.*, 1906, **16**, 20.

[5] Brücke, H. V., *J. Comp. Neur.*, 1931, **53**, 225.

the work of Poos[6] and Ten Cate,[7] who showed that constriction takes place by contraction of the sphincter alone, in contrast to the claim of Behr[8] that the normal constriction of the pupil to light takes place by an active innervation of the sphincter and simultaneous inhibition of the dilator.

Summary. The differences in pupil diameter in the dark adapted rabbit's eye can be summarized as follows: 1. It is smallest when the sympathetic is cut. This state represents an equilibrium between the elasticity of the iris and the "residual tone" of the sphincter. 2. It is slightly larger when both the sympathetic and the third nerves are cut. In this condition, elastic tissue only holds the pupil open. 3. It is still larger in the normal eye when there is an equilibrium between the "residual tone" of the sphincter on the one hand and the elasticity of the iris tissue plus the active contraction of the dilator on the other. 4. It is at its greatest possible maximum when the third nerve is cut or atropine is instilled into the normal eye. Here the "residual tone" of the dilator and the possible slight contraction of this muscle is added to the action of the elastic tissues of the iris.

9957

Oxidative Assimilation of Lactic Acid by *Escherichia coli.*

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Barker,[1] Geisberger,[2] and Clifton[3] have shown that the oxidation of simple substrates by washed suspensions of actively respiring, non-proliferating cells does not proceed to completion, but instead, a portion of the substrate is apparently assimilated by the cells. For example, the oxidative assimilation of acetate by *Escherichia coli* may be represented[3] as

$$2\ CH_3COOH + 3\ O_2 = (CH_2O) + 3\ CO_2 + 3\ H_2O$$

According to this equation three-fourths of the oxygen required for the complete combustion of the acetate is consumed with the production of equivalent amounts of carbon dioxide and water, and at the same time one mol of a substance having the empirical composition of a carbohydrate is formed for each 2 mols of acetate undergoing change. In the presence of appropriate concentrations of sodium azide (NaN_3) the assimilatory process appears to be blocked, the oxidation proceeding to completion. These studies have been extended to include the oxidation of lactic acid in well washed suspensions and in actively proliferating cultures of *Esch. coli,* the rates and extent of oxygen-consumption and of carbon-dioxide production being determined at 30°C by Warburg's technic.

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[6] Poos, F., *Klin. Monatsbl. f. Augen.*, 1927, **78**, 874.

[7] Ten Cate, J., *Arch. Neerl. de Physiol.*, 1921, **6**, 258.

[8] Behr, *Graefe-Saemisch Handbuch der ges. Augenheilkunde*, 1924, **3**.

[1] Barker, H. A., *J. Cell. Comp. Physiol.*, 1936, **8**, 231.

[2] Geisberger, G., *Beiträge zur Kenntnis der Gattung Spirillum Ehbg.*, Dissertation, Utrecht, 1936.

[3] Clifton, C. E., *Enzymologia*, 1937, **4**, 246.

The oxidation of lactate (2 ml, 0.008 M, Fig. 1, A) by *Esch. coli* in a M/15 phosphate buffer of pH 7.1 proceeds at a rapid and quite constant rate until approximately two-thirds of the oxygen required for complete combustion has been consumed. Then the rate of oxidation very rapidly decreases and approaches the level of the substrate-free control. A similar behavior was noted by Cook and Stephenson.[4] In the presence of M/600 or M/400 sodium azide the oxidation proceeds nearer to completion while the theoretical amount (538 μl) of oxygen required for complete combustion of the lactate is consumed in the presence of M/200 azide before the rate of oxidation markedly decreases. These concentrations of azide have little influence on the oxygen-consumption by the substrate-free control, the highest concentration tending to decrease slightly the rate of oxygen-consumption of the blank. Sodium azide, in the concentrations employed, did not appear to exert an appreciably germicidal action under the conditions of these tests. The above results, together with an observed R.Q. of 1.0 suggest that the oxidative assimilation of lactate may be represented as

$$CH_3CHOH \cdot COOH + 2\ O_2 = (CH_2O) + 2CO_2 + 2\ H_2O$$

The influence of 3 different initial concentrations of lactic acid on the rates and extent of oxygen-consumption during the growth of *Esch. coli* in 2 ml of a synthetic medium[5] is illustrated in Fig. 1, B. In the presence of M/300 lactic acid (only source of carbon) the rate of oxygen-consumption broke abruptly and decreased to a low order of magnitude by the time that 280 μl of oxygen had been consumed. This amounts to a consumption of approximately 62% of that required for the complete oxidation of the lactate. Similar breaks in the rates of consumption were observed when 440 and 880 μl of oxygen had been consumed in M/200 and M/100 lactate

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[4] Cook, R. P., and Stephenson, M., *Biochem. J.*, 1928, **22**, 1368.

[5] Clifton, C. E., Cahen, S. F., and Morrow, G., PROC. SOC. EXP. BIOL. AND MED., 1936, **35**, 40.

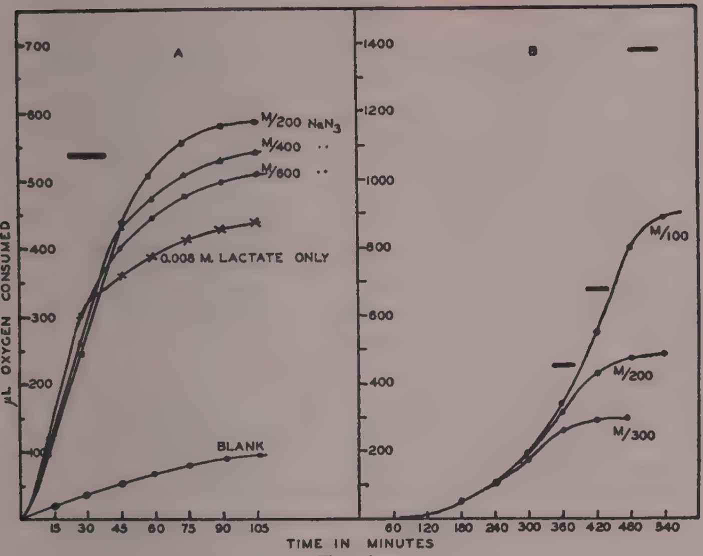

Fig. 1.

A. The influence of different concentrations of sodium azide on the oxidation of 2 ml of 0.008 M lactate by *Esch. coli.*

B. The influence of different initial concentrations of lactate on the rate and extent of oxygen-consumption in cultures of *Esch. coli.* The heavy horizontal lines above the curves represent the theoretical values for the oxygen required for complete combustion of the different amounts of lactate, and are placed immediately above the times at which the substrate is assumed to have been consumed.

medium, respectively, corresponding to an oxygen-consumption of approximately 65% of the theoretical. The rates of carbon dioxide production, and the total amounts of carbon dioxide produced were equal to the corresponding values for oxygen-consumption. The maximal numbers of bacteria developed in these cultures were 59, 81, and 160 × 10^7 per ml in the M/300, M/200, and M/100 lactate cultures, respectively.

Typical values of the amounts of oxygen consumed per ml per hour and per cell per minute together with the numbers of viable bacteria at the end of each interval of time, as observed in a 0.004 M lactate medium, are presented in Table I.

The total oxygen consumed in the experiment outlined in Table I is approximately equal to 66% of that required for the complete combustion of the lactic acid to carbon dioxide and water. In all of the cultures studied not only did the rates of oxygen-consumption and of carbon dioxide production markedly decrease when approximately two-thirds of the lactate had been oxidized, but there was also a marked decrease in the growth-rate at the same time.

TABLE I.
Influence of the Age of a Culture on the Rates of Oxygen-consumption in a 0.004 M Lactate, Inorganic-medium Culture of *Escherichia coli.*

Time, min.	No. of bacteria per ml at end of time interval	μl O_2 consumed by culture per ml per hr	 per cell per min.
0-210	12×10^7	6.0	—
210-300	23 ”	28.0	1.84×10^{-9}
300-360	36 ”	26.0	1.49 ”
360-420	54 ”	42.5	1.60 ”
420-480	72 ”	47.5	1.26 ”
480-540	77 ”	22.5	0.5 ”
540-660	80 ”	10.0	0.1 ”
	Total oxygen consumed	182.5 μl	

This decrease became apparent when approximately 60% of the theoretical amounts of oxygen required for complete combustion had been consumed. The rates of oxygen-consumption observed after 66% of the theoretical amount had been consumed could be accounted for on the assumption that the cells were utilizing reserve cellular material.

The observations reported in this paper lend further support to the hypothesis advanced by Clifton, *et al.*,[5] and by Hershey and Bronfenbrenner[6] that, when oxygen is available in excess, the growth rate and maximal population developed under favorable conditions is primarily controlled by the available foodstuff rather than by any accumulation of inhibitory products. Furthermore, it appears that the equation suggested for the oxidative assimilation of lactic acid by washed suspensions of *Esch. coli* may equally well apply to the assimilation of lactic acid in actively proliferating cultures of this bacterium. Also, the addition of sodium azide to the culture medium proved to be inhibitory to growth, thus suggesting that assimilatory processes are again blocked by the action of the azide. These, and other results to be reported later, suggest that respiration and assimilation are closely connected processes and that the respiration of heterotrophic bacteria may well be represented by the general equation:

Foodstuff + O_2 = Assimilated material + CO_2 + H_2O; much as $CO_2 + H_2O$ + light = $(CH_2O) + O_2$ represents the assimilatory process in the green plant. In the former oxidative type of assimilation, carbon dioxide and water might be regarded as the waste products just as oxygen is the waste product of the assimilatory process in the plant kingdom.

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[6] Hershey, A. D., and Bronfenbrenner, J., PROC. SOC. EXP. BIOL. AND MED., 1937, **36**, 556.

9958

Claim for Thyroid Subnormality in Vitamin E-Low Rats.*

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Singer[1] described a marked hypoplasia of the thyroid in rats 12 to 18 months of age maintained on a vitamin E-low diet.

Barrie[2, 3, 4] observed a similar condition at the end of the lactation period in the young of vitamin E-low mothers; a condition that did not exist when the young were transferred to foster mothers receiving a stock ration.

Relatively large numbers of young are occasionally produced in this laboratory as a result of "first litter fertility," in mothers reared and held on E-low diets. There are good reasons for believing that in these cases of fertility, the mother possesses stores of vitamin E barely sufficient for the birth of living young. The reasons are:

1. The majority of the mothers of a group handled at the same time were sterile.
2. On rebreeding, these females invariably resorb.
3. A high proportion of the young from "first litter fertilities" show a characteristic deficiency picture toward the end of the lactation period consisting of: the paralysis described by Evans and Burr,[5] a characteristic configuration of the head, general puffiness, roughness of fur in spite of normal body weight, a corneal film, etc.

Young from 3 differently treated groups of rats were examined by us.

Group 1. The mothers were reared on vitamin E-low diet 427[6] and were bred at the first pro-estrus after 60 days. Each mother was allowed to suckle 6 young, and at 10 days a part of each litter

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We wish to acknowledge assistance rendered by the Federal Works Progress Administration, Project No. 7787.

The following materials were generously contributed: cod liver oil by E. R. Squibb & Sons, brewers' yeast by the Vitamin Food Company, New York, and wheat germ oil by General Mills, Inc., Minneapolis.

[1] Singer, E., *J. Physiol.*, 1936, **87**, 287.

[2] Barrie, M. M. O., *Nature*, 1937, **139**, 286.

[3] Barrie, M. M. O., *Nature*, 1937, **140**, 426.

[4] Barrie, M. M. O., *Lancet*, 1937, **2**, 251.

[5] Evans, H. M., and Burr, G. O., *J. Biol. Chem.*, 1928, **76**, 273.

[6] Emerson, G. A., and Evans, H. M., *J. Nutrition*, 1937, **14**, 169.

was given 2 drops of a standardized wheat germ oil 6 times weekly. There were 24 animals in the untreated group and 19 in the treated.

Group 2. Vitamin E-low females that had undergone a typical resorption gestation were given 1 g of wheat germ oil on the day of the second positive mating. Six young from each litter were kept, of which three were suckled by the mother and the remaining 3 were given to a foster mother that received an additional 1 g of wheat germ oil on the day of littering. Three young from the foster mother were in turn given to the original mother.

Group 3. Mothers with a history of a resorption gestation were handled as in group 2 except that half of the young from each mother received by stomach tube from day 5 or 10, 0.2 cc of wheat germ oil. There were 17 untreated and 14 treated animals.

Group 4. Six normal young, 21 days of age, from the stock colony.

The size of the thyroid vesicles, their content of colloid and especially the height of the epithelium were items charted for each animal in all groups. Characteristic differences between the groups were not observed. Variations within the group fully equalled the group differences claimed by Singer and Barrie.

If vitamin E deficiency imposes subnormality on the thyroid, increased evidence of this might legitimately be expected in older animals, in those reared and held for their entire life on a vitamin E-low ration. Two groups of animals were observed: 6 rats that were 22 months of age and had been maintained on a vitamin E-low ration since weaning, and 5 controls of the same age reared on a stock diet. As in the case of the suckling young, these animals showed no histological evidence of hypothyroidism.

It occurred to us as entirely possible that functional subnormality might be detectable even with histological normality; consequently a preliminary study was made of the oxygen consumption of 4 vitamin E-low, and 4 normal, rats. No significant differences were observed, the average figure being slightly higher for the experimental than for the control group.

Conclusion. Histological subnormality of the thyroid was not observed in the paralyzed suckling young of vitamin E-low mothers or in old animals (22 months of age) chronically deprived of vitamin E.

9959 P

Degeneration of Cross Striated Musculature in Vitamin E-Low Rats.*

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It has been noted for some years in this laboratory that animals reared and held upon E-low rations develop characteristic disability in locomotion, or partial paralysis accompanied by obvious, naked-eye atrophy of some of the musculature of the flank and posterior limb, particularly adductors of the leg. Because of convenience, we have submitted certain muscles of the leg (gastrocnemius and soleus) of these animals to microscopic examination, although these were not necessarily the muscles most affected. This note is to report the invariable occurrence of degenerative lesions of the gross striated musculature in adult rats that had been placed upon vitamin E-low rations at weaning and autopsied at 22 months. The paralysis was mentioned in a summary by Evans.[1] It was later mentioned by Ringsted[2] and by Burr, Brown and Moseley.[3] A similar condition in young animals was described by Evans and Burr,[4] and later by Olcott,[5, 6] who have reported microscopic changes strikingly similar to those observed in muscular dystrophy in herbivora and to those herein described.

Six animals were placed at weaning (21 days) on vitamin E-low diet 427[7] (5 controls of the same age were put on stock diet I), and held there until the time of autopsy at 22 months. The animals in both groups had a history of one breeding. The disability in locomotion was usually evident by the 15th month. At the time of

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* Aided by grants from the Research Board and the College of Agriculture of the University of California, the Rockefeller Foundation of New York, and Merck & Company, Inc., Rahway, New Jersey.

We wish to acknowledge assistance rendered by the Federal Works Progress Administration, Project No. 7787.

The following materials were generously contributed: cod liver oil by E. R. Squibb & Sons, and brewers' yeast by the Vitamin Food Company, New York.

[1] Evans, H. M., *J. A. M. A.*, 1932, **99**, 469.

[2] Ringsted, A., *Biochem. J.*, 1935, **29**, 788.

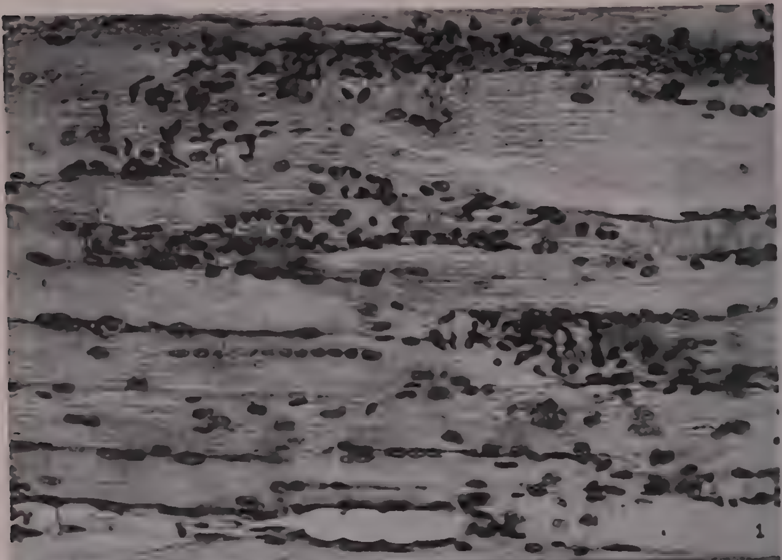
[3] Burr, G. O., Brown, W. R., and Moseley, R. L., PROC. SOC. EXP. BIOL. AND MED., 1927, **36**, 780.

[4] Evans, H. M., and Burr, G. O., *J. Biol. Chem.*, 1928, **76**, 273.

[5] Olcott, H. S., Proc. Am. Soc. Biol. Chem., *J. Biol. Chem.*, 1937, **119**, xxiv.

[6] Olcott, H. S., *J. Nutr.*, 1938, **15**, 221.

[7] Emerson, G. A., and Evans, H. M., *J. Nutr.*, 1937, **14**, 169.



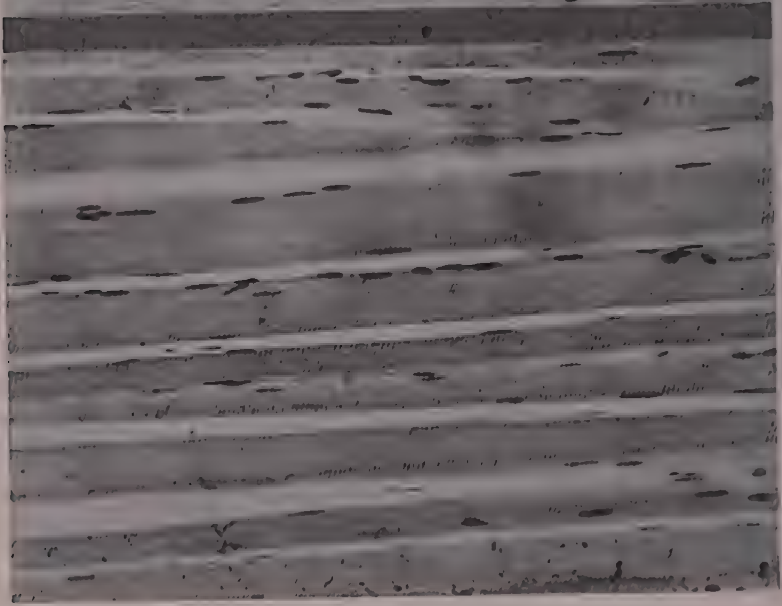


FIG. 1.

Section of gastrocnemius muscle from a paralyzed vitamin E-low rat 22 months of age.

FIG. 2.

Section of gastrocnemius muscle from a normal rat 22 months of age.
Slides stained with hematoxylin and eosin × 365.

sacrifice the animals weighed on the average almost 100 g less than the controls (252:335 g) and on contrast to the smooth, normal pellage which still characterized the controls, their sparse pellage gave a rough appearance, and in places complete denudation occurred. This was particularly the case on the back in the region of the flanks, where naked-eye atrophy of the musculature was apparent. There was also invariably an accelerated respiratory rate as contrasted with normal animals. The animals were sensitive, irritable creatures and were not easily handled.

Histological Findings. Sections of the gastrocnemius were fixed in Bouin's or Zenker formol. After nitro-cellulose embedding, the tissues were sectioned at 6-8μ and stained with hematoxylin, eosin or iron hematoxylin, aniline blue.

A marked infiltration of leucocytes and connective tissue elements was noted. Cross striations were usually reduced, and in some cases were entirely lacking. Many of the muscle and sarcolemma nuclei were shrunken and pyknotic, staining deeply with hematoxylin.

Within the sarcolemma, multiplication of nuclei often occurred; the nuclei were arranged in rows parallel to the long axis of the fiber. The chains of nuclei, in some instances, persisted after the necrotic fibers had been resorbed and the sarcolemma collapsed. The degenerating fibers were often replaced by fat and other connective tissue elements.

The iron-hematoxylin aniline blue stain showed areas where within the sarcolemma there was a failure of both longitudinal and transverse striations and chains of contiguous nuclei. These areas in the sarcoplasm stained light blue with the stain, in contrast to the pink of healthy fibers. In other instances the entire muscle fiber was replaced by a foamy, light blue sarcoplasm with many nuclei.

Conclusion. Muscular dystrophy characterizes old rats reared and maintained on diets devoid of vitamin E. The histological lesions are outstanding.

9960 P

Skin Vascular Reactions to the Cold Pressor Test.

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The detection of the presence or absence of hyperreactivity of the vasomotor system by the standard cold stimulus used by Hines and Brown and others,[1] depends on statistically significant differences in the arterial blood pressure responses. Although the conclusions of Hines and Brown have been challenged,[2] a systematic study of the vasomotor reactions involved would seem to be desirable for a more complete analysis of the significance and mechanism of the test.

The development of the skin photoelectric plethysmograph[3] offered opportunity to explore the participation of the skin vessels in the responses to the immersion of one hand in ice-cold water. Responses in the finger (recorded with a mechanical plethysmograph), ear and forehead (recorded photoelectrically) have been observed in a random group of 9 normal male subjects (6 whites, 2 Negroes, 1 Chinese) in 24 experiments. The number of observations is too few to bear on the significance of the cold pressor test. They are not offered for that purpose.

Constriction in the finger of the opposite hand was observed in all trials in all subjects. This is a well-known and frequently demonstrated response to the immersion of a limb in cold water. In contrast to the behavior of the finger vessels, the responses in the ear and forehead varied from a slight decrease in blood volume through no effect to an increase in blood volume. Similar changes were observed in the amplitude of the volume pulse. In some cases, a decreased volume pulse was recorded when no decrease in blood volume occurred.

The interpretation of these skin changes as of vasomotor origin is complicated by the fact that the blood content of the skin of the head is readily altered by changes in intrathoracic pressure. The respirations often change considerably as a result of the pain of the test. More prolonged or "active" expirations or shift of the chest to a more expiratory position tend to engorge the venous vessels of the head skin. These effects will obscure a simultaneous slight constriction in this area. Decrease in the amplitude of the volume pulse may result from increased intrathoracic pressure as well as from arterial constriction so that in those instances where the pulse decreased (the changes were slight) this cannot be considered as evidence of constriction. It is, therefore, suggested that in normal subjects the skin vessels of the head participate only slightly (if they do at all) and to a much less extent than is true for the finger vessels, in the vasoconstrictor response to the cold pressor test.

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[1] Hines, E. A., Jr., and Brown, G. E., *Ann. Int. Med.*, 1933, **7**, 209; *Am. Heart J.*, 1936, **11**, 1; White, B. V., and Gildea, E. F., *Arch. Neurol. and Psychiat.*, 1937, **38**, 964.

[2] Yates, M. R., and Wood, J. E., Jr., Proc. Soc. Exp. Biol. and Med., 1936, **34**, 560.

[3] Hertzman, A. B., Proc. Soc. Exp. Biol. and Med., 1938, **38**, 562.

9961 P

Effect of Potassium Chloride on the Normal and Denervated Iris.

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Camp and Higgins[1] have called attention to the similarity of the actions of epinephrin and the potassium salts on various organs and have advanced the hypothesis that epinephrin acts by liberating potassium; no reference was made by them to the action of potassium on the iris.

Experiments were carried out using intact and excised bulbi of frogs and intact normal and denervated eyes of living rabbits. Forty-four excised frog bulbi placed in 0.6% KCl, and observed 90 to 150 minutes, resulted in 32 constrictions, 1 dilation, and 11 showing no change. Control eyes placed in 0.6% NaCl gave similar results.

In another sseries of experiments, the cerebra of frogs were pithed or lightly crushed and 0.25 to 1 cc 0.6% KCl injected intracardially or into the aorta. Of 66 eyes observed, 18 showed no change; 41 constricted and 7 dilated. The dilatations were slight or moderate, the maximum being 1 mm. Control injections of 0.6% NaCl produced constriction or no change.

Large doses of KCl, *i. e.*, 1 to 2 cc of a 4.0% solution injected intraärterially into 12 frogs produced constriction in all cases. During the constricted state 0.25 to 0.5 cc epinephrin 1:10,000 was injected intracardially or into the aorta; in all cases the pupils markedly dilated.

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[1] Camp, W. J. R., and Higgins, J. A., *J. Pharm. Exp. Therap.*, 1936, **57**, 376.

Seventeen excised bulbi from frogs previously injected with 1-2 cc 1.0% KCl showed no greater sensitivity to dilute solution of epinephrin than 18 control eyes from frogs injected with similar amounts of NaCl.

Three series of experiments were carried out on living rabbits having one iris sympathectomized, by removing the superior cervical ganglion.

In the first series, 11 experiments on 6 rabbits, 1.0 cc of a 4.1% solution of NaCl was injected subconjunctivally in both eyes. There were no changes except for a few transitory constrictions.

In a second series, 11 experiments on 5 animals, 1.0 cc of sterile 5.0% KCl was injected subconjunctivally in both eyes. Marked constriction occurred in both eyes in all cases, with approximate return to normal in 70-80 minutes. In one experiment, the pupils remained constricted for over 2 hours. At no time in any of the experiments was there definite dilatation above normal.

In the third series, 14 experiments on 6 rabbits, 1.0 cc sterile 5.0% KCl was injected subconjunctivally into both eyes. Early during the constricted state, 0.5 cc epinephrin (1:10,000) per kilo body weight was injected intramuscularly. In all, except one experiment, there was a definite dilation on the sympathectomized side. On the normal side, no definite dilatations were noted; the pupils returned gradually from a constricted state to normal.

Control injections of epinephrin 1:10,000 intramuscularly produced dilatations on the sympathectomized sides with no apparent effect on the normal sides; the dilatations were no greater than in the KCl-epinephrin series.

In none of the experiments on frogs or rabbits was any similarity found between the actions of potassium and epinephrin. KCl in the concentrations used constricts the pupil of intact and excised eyes of frogs and the normal and sympathectomized iris of rabbits. Epinephrin overcame the constrictions so produced even when the dosages of potassium were excessive. If KCl exerts an epinephrin-like effect only at certain concentrations, one would expect the optimum concentration to be obtained at some time during the absorptive process.

The above observations do not support the hypothesis that epinephrin acts by liberating potassium.

9962 P

Arginase in the Mammary Gland.

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The liver has been considered as the only gland in the mammalian body capable of synthesizing urea. Hunter and Daupinee[1] reported that the liver contained large quantities of arginase which has not been reported for other glands in the mammalian body. Krebs and Henseleit[2] advanced the generally accepted hypothesis that arginase is one of the essentials in the formation of urea.

Since urea has been reported to be produced by the mammary gland by Graham, Houchin and Turner,[3] it appeared probable that the method of formation was similar to that in the liver and that arginase may be present in the mammary gland.

Accordingly, both lactating and non-lactating mammary glands from cows were analyzed for arginase by the method described by Hunter and Daupinee.[4] In each case 100 g of udder tissue were extracted with 100 ml of 75% glycerol. For the trials 5 ml of the glycerol extract were added to the arginine solution and the urea formed after 24 hours of incubation was determined by the urease method of Van Slyke and Cullen.[5] Blanks were run identically with

TABLE I.
Urea Nitrogen Formed by Mammary Gland Extract.

Cow No.	Nitrogen as arginine HCl, mg	Urea nitrogen found in blank, mg	Total urea nitrogen found, mg	Remarks
1	6	.36	.80	Lactating glands
2	6	.13	.31	
3	6	.04	.10	
4	6	.22	.35	
5	6	.41	.78	
6	6	.28	.62	
7	6	.43	.92	
8	6	.24	.27	Non-lactating glands
9	6	.22	.26	
10	6	.00	.00	

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[1] Hunter, A., and Daupinee, J. A., *Proc. Roy. Soc. London*, 1925, **97**B, 227.

[2] Krebs, H. A., and Henseleit, Kurt, *Hoppe-Seyler's Z. Physiol. Chem.*, 1932, **210**, 33.

[3] Graham, W. R., Houchin, O. B., and Turner, C. W., *J. Biol. Chem.*, 1937, **120**, 29.

[4] Hunter, A., and Daupinee, J. A., *J. Biol. Chem.*, 1929, **85**, 627.

[5] Van Slyke, D. D., and Cullen, G. E., *J. Biol. Chem.*, 1916, **24**, 117.

the exception that arginine was omitted. The urea nitrogen in the blanks varied from 0.0 mg in the case of the dry glands to as much as 0.43 mg in the case of lactating glands. The amounts of urea nitrogen from 5 ml of glycerol extract as compared to 5 ml of glycerol extract and arginine hydrochloride are given in Table I.

From these data it is obvious that the active mammary gland contains appreciable arginase while the inactive gland contains less or none at all as revealed by the methods used.

It will be noted in the table that the amount of arginase in the mammary gland extract is proportional to the amount of preformed urea in the mammary gland extract, as shown by the blank determinations. This urea was probably formed in the gland or gland extract by arginase and urea precursors in the gland. Additional controls demonstrated that the arginine used did not contain free urea.

9963

Amino Acids and other Non-Protein Nitrogen Blood Substances in Relation to Milk Secretion.

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Since Cary[1] demonstrated that the amino-acid content of blood obtained from the mammary vein of lactating cows was significantly lower than that obtained from the jugular vein, it has been assumed that the amino-acids of the blood are the precursors of the milk proteins. Recently Graham,[2] and Shaw and Petersen[3] have presented evidence that the blood amino-acids are not the sole precursors of milk proteins. Graham, *et al.*,[4] have also demonstrated that the blood proteins may be involved in the protein metabolism of the mammary gland.

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* The data in this paper are from a thesis presented by J. C. Shaw in partial fulfillment of the requirements for the degree of Doctor of Philosophy, University of Minnesota, Minnesota Agricultural Experiment Station.

[1] Cary, C. A., *J. Biol. Chem.*, 1920, **43**, 477.

[2] Graham, W. R., Jr., *J. Biol. Chem.*, 1937, **122**, 1.

[3] Shaw, J. C., and Petersen, W. E., *Proc. Am. Physiol. Soc.*, March 30, 31, April 1, 2, 1938, 50th Annual Meeting, Baltimore, Maryland, p. 183.

[4] Graham, W. R., Jr., Peterson, V. R., Houchin, O. B., and Turner, C. W., *J. Biol. Chem.*, 1938, **122**, 275.

Data are presented in this paper which show that the amino-acids of the blood cannot account for more than 30 to 40% of the milk proteins.

Arterial and mammary venous bloods were obtained and handled as reported by Shaw, Boyd and Petersen.[5]

Amino-acids were determined in tungstomolybdic acid filtrates by the colorimetric method of Folin.[6] The apparent creatine and creatinine in blood were determined on the same filtrate by the method of Folin and Wu.[7] Uric acid was determined on blood plasma by Benedict's direct colorimetric method.[8] The Clark-Collip[9] modification of the Kramer-Tisdall method was employed for the determination of calcium. Urea was determined on blood plasma samples by the urease method of Van Slyke and Cullen.[10, 11]

Cary[1] made no attempt to determine the quantity of amino-acids which would have to be removed by the mammary gland to account for all of the proteins of the milk. In order to obtain a more complete picture of the nitrogen metabolism of the gland several non-protein fractions were determined on simultaneously drawn arterial and mammary venous bloods. Of these, herein are reported amino-acids, uric acid, urea, creatine and creatinine. Data are presented in Table I comparing the amino-acid and calcium arterio-venous difference in the passage of the blood through the mammary gland.

Amino-acids were removed from the blood by the gland in fairly constant amounts with an extreme variation of from 0.15 mg % to 0.97 mg %. The average arterio-venous difference was 0.46 mg %. On the basis of the protein in the milk this indicates that approximately 1200 volumes of blood per unit volume of milk would be necessary to supply enough amino-acids to account for the milk proteins if the amino-acids were the sole precursors of milk proteins. The average calcium loss of 0.32 mg % indicates that the volume flow of blood per unit volume of milk is approximately 390.

This is in good agreement with similar ratios calculated from fat and carbohydrate A-V differences and the quantity of fat and carbohydrate in the milk, Shaw and Petersen.[3] It is evident then that the amino-acid absorption by the mammary gland is much too small to

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[5] Shaw, J. C., Boyd, W. L., and Petersen, W. E., PROC. SOC. EXP. BIOL. AND MED., 1938, **38**, 579.

[6] Folin, O., *J. Biol. Chem.*, 1922, **51**, 377.

[7] Folin, O., and Wu, H., *J. Biol. Chem.*, 1919, **38**, 81.

[8] Benedict, S. R., *J. Biol. Chem.*, 1922, **51**, 187.

[9] Clark, E. P., and Collip, J. B., *J. Biol. Chem.*, 1925, **63**, 461.

[10] Van Slyke, D. D., and Cullen, G. E., *J. Biol. Chem.*, 1914, **19**, 211.

[11] Van Slyke, D. D., and Cullen, G. E., *J. Biol. Chem.*, 1916, **24**, 117.

TABLE I.
Amino Nitrogen in Simultaneously Drawn Samples of Arterial and Mammary Venous Bloods of Cows as Related to Calcium A-V Differences.

Amino nitrogen level			Plasma calcium	
Arterial, mg %	Venous, mg %	A-V difference, mg %	A-V difference, mg %	Remarks
3.92	3.25	—0.66	—0.31	2 hr. after milking
4.38	3.86	—0.52	—0.24	
3.72	3.26	—0.46	—0.54	
3.85	3.36	—0.49	—0.35	
3.08	2.93	—0.15	—0.19	3 ” ” ”
4.23	3.72	—0.51	—0.30	
3.33	3.04	—0.29	—0.20	
3.76	3.23	—0.53	—0.32	4 ” ” ”
3.30	3.05	—0.25	—0.46	
3.90	3.25	—0.65	—	
4.49	4.08	—0.31	—0.51	9 ” ” ”
4.40	4.09	—0.31	—	
4.13	3.97	—0.16	—0.14	
4.28	3.31	—0.97	—0.29	
3.53	3.51	—0.02	—0.07	dry cow
Avg of lactating cows				
3.90	3.44	—0.46	—0.32	

account for all of the milk proteins. If all of the amino-acids absorbed by the mammary gland from the blood were converted into milk proteins there would still be about 65% of the milk proteins to be accounted for by some other nitrogen substances in the blood. Graham[2] conducted a carbohydrate balance experiment on the mam-

TABLE II.
Uric Acid in Simultaneously Drawn Samples of Arterial and Mammary Venous Bloods.

	Uric acid level		
Cow No.	Arterial, mg %	Venous, mg %	Arterial Venous Difference, mg %
241	1.50	1.32	—.18
445	1.72	1.30	—.42
235	1.39	1.34	—.05
432	1.50	1.65	+.15
447	1.64	1.55	—.09
419	1.79	1.72	—.07
433	1.80	1.73	—.07
23	1.43	1.36	—.07
25	1.41	1.41	0
A-7	1.76	1.82	+.06
586	1.75	1.79	+.04
Avg	1.60	1.55	—.05

mary gland and reported 2 observations on amino-acids in which the amino-acid loss when compared to the glucose and lactic acid loss appeared to be too small to account for the milk proteins. In a later paper Graham, *et al.*,[4] presented experimental data indicating that the blood proteins were involved in the protein metabolism of the mammary gland.

The fact that the amino-acids cannot account for the major portion of the milk proteins lends support to the hypothesis that the blood proteins are involved in the elaboration of milk proteins.

The data presented in Table II show that only very small amounts of blood uric acid are removed by the lactating mammary gland. In 11 analyses there was an average uric acid loss of –0.05 mg %.

Data are given in Table III on the arterio-venous creatinine and creatine differences. While there was a loss of creatinine and creatine in the majority of cases, the amounts absorbed by the mammary gland were extremely small. The average loss of each was 0.06 mg %.

TABLE III.

"Apparent" Creatine and Creatinine in Simultaneously Drawn Samples of Arterial and Mammary Venous Bloods.

	Creatinine level		Arterial Venous Difference, mg %	Creatine level		Arterial Venous Difference, mg %
Cow No.	Arterial, mg %	Venous, mg %		Arterial, mg %	Venous, mg %	
443	1.29	1.20	—.09	2.23	2.16	—.07
445	1.56	1.35	—.21	1.63	1.75	+.12
235	1.31	1.28	—.03	1.73	1.73	0
432	1.49	1.45	—.04	2.12	2.06	—.06
447	1.37	1.35	—.02	2.23	2.04	—.19
577	1.07	1.07	0	2.07	1.88	—.19
434	1.25	1.21	—.04	2.03	2.04	+.01
241	1.20	1.12	—.08	—	—	—
419	1.27	1.25	—.02	—	—	—
440	1.22	1.16	—.06	—	—	—
Avg	1.30	1.24	—.06	2.01	1.95	—.06

Conclusions. 1. The amino-acids absorbed from the blood by the mammary cannot account for more than 35% of the milk proteins. 2. While there is a slight loss of blood uric acid, creatine and creatinine to the lactating mammary gland, it is evident that these substances are not important in the nitrogen metabolism of the lactating gland.

9964 P

Influence of Induced Variations in Electrolyte and Water Exchanges with Pitressin in Bronchial Asthma.*

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During the course of studies on the influence of various therapeutic procedures on the mineral and water exchanges in children suffering from bronchial asthma, it was observed that artificial fever was usually followed by remission of symptoms when the sodium chloride content of the diet was extremely low, but not when the intake of this salt was unrestricted. This observation suggested the desirability of examining more critically the relationship between the electrolyte and water balance in the mechanism of asthmatic attacks.

Starling and Verney[1] and McQuarrie, Manchester and Husted[2] have shown that pitressin, the antidiuretic principle of the pituitary gland causes, not only a retention of body water, but a coincident absolute increase in the output of sodium and chloride in the normal subject. Therefore, when the sodium chloride intake is very low and the water intake is relatively high during a period of sustained pituitary antidiuresis, body water is retained without coincident storage of sodium chloride. Since this procedure offers a unique opportunity to dissociate the effects of water and salt in the asthmatic patient, the following experiments were undertaken.

Six children, selected on the basis of severity and intractibility of their asthmatic attacks, were placed under constant environmental conditions in the hospital, in charge of specially instructed nurses. The patients were given 4 evenly spaced daily feedings of a weighed formula having reasonably adequate basal dietary constituents, but an extremely low sodium content. Calculated daily intake of sodium was 120 to 700 mg. Distilled water was given at equally spaced intervals, 400 cc per day during control periods and 1200 cc to 2000 cc per day during pitressin sessions. Urine was collected in 24-hour specimens, measured and preserved for future chemical analysis. The subjects were weighed daily on a metabolism balance

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* This work was supported by a grant from the Medical Research Fund of the University of Minnesota.

[1] Starling, E. H., and Verney, E. B., *Proc. Royal Soc. Biol.*, 1925, **97**, 321.

[2] McQuarrie, Irvine, Manchester, R. C., and Husted, Clara, *Am. J. Dis. Child.*, 1932, **43**, 1519.

under uniform conditions. Hourly observations of the patients' condition were made and all asthmatic attacks were tabulated.

An initial control period accustomed the children to the regimen and established the frequency and severity of the asthmatic attacks. Then, while the low salt and high water intakes were maintained, pitressin was administered by hypodermic injection in doses of 0.3 to 0.5 cc every 3 hours which had been found by McQuarrie and Peeler[3] to be sufficient to sustain antidiuresis over a considerable period of time. After 24 to 36 hours, during which the weight increased due to retained water 2 to 5% of the initial body weight,

TABLE I.
Influence of Pitressin on Attacks of Bronchial Asthma.

		Intake mg per day		Amt of asthma during various periods*			
Case	Exp. No.	Na	K	Control	Pitressin	Diuresis	Subsequent
I.	1	120	360	4	1	1	3
H.B.	2	120	360	4	1	1	2
	3	120	360	4	2	2	0
	4	120	360	1	0	0	0
II.	5	120	360	1	0	0	0
M.M.	6	200	600	3	0	0	0
	7	200	2100	3	0	0	0
	8	200	2100	3	1	0	0
	9	200	2100	2	1	0	0
	10	500	1500	3	1	0	0
	11	500	1500	1	0	0	0
	12	200	600	4	2	0	0
III.	13	500	1500	2	0	0	0
R.K.	14	200	600	3	0	0	0
	15	200	600	2	3†	4†	1
IV.	16	700	2000	4	2	1	1
R.F.	17	700	2000	3	3	2	1
V.	18	120	360	4	2	0	0
B.C.	19	120	360	2	1	0	0
VI. R.V.	20	200	600	4	1	0	0

*Amount of asthma was determined by hourly observations day and night and number of doses of epinephrine required to give the patients some comfort. The following code was adopted:

0—No asthmatic wheezing observed and no epinephrine used.
1—Wheezing present for at least 1 hr per day requiring 1 dose of epinephrine.
2—Wheezing lasted 3 hr per day requiring usually 3 doses of epinephrine.
3—Wheezing present about half the time or at least 12 hr per day, requiring around 5 doses of epinephrine per day.
4—Continuous wheezing existed requiring 8 or more doses of epinephrine per day.

†Vomiting, cramps, and diarrhea were present during these periods and the asthma was temporarily aggravated.

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[3] McQuarrie, Irvine, and Peeler, D. B., *J. Clin. Investigation*, 1931, **10**, 915.

the pitressin and extra water were stopped. This resulted after 6 hours in a copious diuresis, the patients usually reaching a new weight level below the initial figure.

A total of 20 observations or studies were made on the 6 subjects. During the water retention phase of each period of observation a reduction in the number and severity of asthmatic attacks occurred in 18 instances, 7 of which showed complete cessation of symptoms. In one case the symptoms remained unchanged and in another child who developed diarrhea a slight increase in symptoms occurred. During and after the diuretic phase improvement in symptoms continued in 19 instances with complete remissions developing 14 times. In the case which had diarrhea the asthma continued to be slightly aggravated during diuresis but improved during the subsequent period. In 6 instances symptoms recurred spontaneously after the end of the period of observation, while the remainder had remissions until upset by some extraneous factor. In one case 4 g of sodium chloride were administered after 10 days, which were free of asthma, all other factors remaining constant, and the asthmatic symptoms reappeared promptly. The results of the experiments are summarized in Table I.

From the foregoing it would appear that bronchial asthma can be ameliorated even in the presence of excessive hydration if there is an associated depletion of sodium chloride. This preliminary group of observations suggests that the sodium ion may exercise an adverse influence on the asthmatic patient, independent of its usual relation to hydration.

9965

Studies on Enzymatic Formation and Destruction of Uric Acid in Mammalian Blood.

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The colorimetric estimation of uric acid by a phosphotungstic acid reagent is made more specific by the use of uricase. The uricase preparation used consists of ox-kidney which has been acetone-dehydrated, benzene-defatted, air-dried, and finely ground. In the studies described here we used the direct colorimetric method[1] that has been in use in this laboratory for a number of years. The cyanide solution described in this method is replaced by a solution of 2.5% sodium cyanide in 25% urea, and the tubes are kept at room temperature for 3 hours instead of being heated to allow the color to develop. Two-cc quantities of blood are incubated for 2 hours at 40° to 48°C. with 50-mg samples of uricase, after which the blood proteins as well as the uricase are precipitated with tungstic acid. The uric acid color-value of this filtrate is determined and subtracted from the value obtained on a filtrate from untreated blood—the difference represents true uric acid.

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[1] Koch, F. C., *Practical Methods in Biochemistry*, 2nd edition, Wm. Wood, Baltimore (1937), 130, 284.

The average value obtained on 25 samples of human blood before uricase action was 3.0 mg % (varying from 1.9 to 4.6) and the average value of these same samples treated with uricase was 0.8 mg % (from 0.7 to 1.1). The true uric-acid value for these samples, then, is 2.2 mg % (varying from 1.1 to 3.8). Recoveries on 15 samples of human blood to which uric acid had been added in quantities of from 2 to 16 mg % averaged 92% (varying from 82 to 100%). The uric acid added to blood was quantitatively destroyed by uricase, leaving the same non-uric acid color-value as that of the original blood.

A typical sample of dog blood gave the following results: The uric-acid color-value on the untreated blood was 2.1 mg % and on the uricase-treated blood, 1.8 mg %, giving 0.3 mg % as the true uric-acid value. The true uric-acid value obtained on 10 samples of dog blood ranged from 0 to 0.5 mg %. Uric acid added to dog blood is recovered with the same accuracy as with human blood, and is quantitatively destroyed when uricase is allowed to act on it, showing that there is nothing in dog blood to prevent the detection of uric acid by this method.

The blood of white normal adult rats has a uric-acid value comparable to that of human blood when it is first drawn, but this value rises slowly as the blood stands at refrigerator temperature, and more rapidly when it is heated at 40° to 48°C. Uricase destroys the uric acid present in the original blood and that formed on incubation of the blood. This formation of uric acid was observed with blood drawn and kept sterile as well as with blood collected without any attempt to use sterile procedures.

A typical experiment showing the increase in uric acid as rat blood stands at refrigerator temperature follows: A pooled sample of blood was drawn by heart puncture from 10 adult normal rats. Oxalate was used to prevent coagulation. Immediately after the last sample was drawn which was about one-half hour after the first sample, the blood contained 1.5 mg % uric acid before uricase action and 0.7 mg % after uricase action, making a true uric-acid value of 0.8 mg %. After the blood had stood for 2 hours this value had risen to 2.2 mg % (2.7 mg % before uricase and 0.5 mg % after uricase action). After 7 hours, the true uric-acid value was 2.5 mg %; after 26 hours, 3.4 mg %; after 4 days, 5.9 mg %; and the final analysis after 8 days showed a uric-acid content of 7.6 mg %. The non-uric-acid color-value in all the cases was from 0.4 to 0.6 mg %.

Different samples of blood vary in their original uric-acid content, but all show approximately the same increase in uric acid with time. This increase is from 2.5 to 3.0 mg % in 24 hours and 7.0 mg % in 8 days at refrigerator temperature. Blood incubated for 2 to 3 hours at 40° to 48°C shows a formation of uric acid of the same order as that produced in the blood standing 24 hours in the refrigerator. The production of uric acid is not observed when the blood proteins are precipitated, the uric-acid content of the filtrates remaining the same for the duration of the experiment.

In an attempt to explain the formation of uric acid on incubation of rat blood, the blood was incubated with adenine, guanine, hypoxanthine, and xanthine. Both guanine and xanthine caused a tremendous uric-acid formation. A typical experiment follows: Rat blood that had stood at room temperature for 4 hours after it was drawn showed a uric-acid content of 5.7 mg %, and after incubation at 40° to 48°C the value increased to 8.5 mg %. Since all the purines used were dissolved in alkali it was necessary to run a control with alkali. The value in this case was 9.2 mg %. The uric acid value of blood incubated with adenine was 7.2 mg %; with hypoxanthine, 7.3 mg %; with guanine, 32 mg %, and with xanthine, 40 mg %. In every case the uric acid produced was completely destroyed by uricase, the non-uric-acid color-value for this experiment being 1.1 mg %. The incubation of blood and uricase with xanthine or guanine either prevented the formation of uric acid or destroyed it as fast as it was formed. In each case in this experiment, 1 cc of blood was incubated with 1 mg of the purine. In this instance 1 cc of blood formed 0.2 mg of uric from 1 mg of guanine and 0.3 mg uric acid from 1 mg of xanthine in 4 hours at 40° to 48°. When 0.1 mg of hypoxanthine is incubated with 1 cc of blood, it also is converted into uric acid.

Neither human nor dog blood showed any tendency to form uric acid on incubating the blood alone, the values on these bloods remaining constant for a period of 5 days at refrigerator temperature.

No uric acid was formed by incubating these bloods with the purines used with rat blood.

Rat blood then contains enzymes which convert guanine, xanthine, and hypoxanthine into uric acid. The nature of the action suggests the presence of guanase and a very active xanthine oxidase.

Dixon and Keilin[2] reported the inhibition of xanthine oxidase by cyanide. The production of uric acid on incubation of rat blood alone or with guanine or xanthine was inhibited by 0.01 M potassium cyanide. It was of interest to find that the quinimine form of para-amino-phenol reported by Bernheim and Bernheim[3] as an inhibitor for xanthine oxidase also inhibited the formation of uric acid from xanthine by rat blood.

9966

Vitamin C and Peptone Shock in Dogs.

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Hochwald[1] and others have reported that the administration of Vitamin C to sensitized guinea pigs exercises a protective effect against the anaphylactic reaction produced by the provocative injection of the antigen. Hochwald reported that Vitamin C administration had no protective effect against histamine shock and therefore postulated that the mechanism of the action of Vitamin C in anaphylaxis was to prevent the liberation of histamine rather than to inhibit or inactivate the action of histamine after its liberation. Ungar, Parrot, and Levillain[2] reported some *in vitro* experiments tending to confirm this hypothesis. We were unable to confirm the protective effect of Vitamin C against anaphylaxis in the dog. Since the anaphylactic experiment is, however, quite complex and it is not always possible to discriminate between those agents which may interfere with the antibody-antigen reactions and those which modify the resultant reaction, it seemed desirable to test the above hypothesis of the mechanism of action of Vitamin C in a histamine liberating reaction that is apparently not dependent upon an antigen-antibody reaction. Such a reaction occurs in peptone shock. We have demonstrated that peptone shock in dogs is accompanied by an explosive liberation of histamine and that the degree of shock is proportional to the amount of histamine liberated.[3] We have, therefore, studied the effect of the prior administration of Vitamin C upon the severity of peptone shock in dogs.

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[2] Dixon, M., and Keilin, D., *Proc. Roy. Soc. London*, 1936, **119**B, 159.

[3] Bernheim, F., and Bernheim, M. L. C., *J. Biol. Chem.*, 1938, **123**, 307.

[1] Hochwald, A., *Z. f. d. ges. exp. Med.*, 1935, **97**, 433.

[2] Ungar, G., Parrot, J. L., and Levillain, A., *C. R. Soc. de biol.*, 1937, **125**, 1015.

Eleven dogs were anesthetized with ether and sodium barbital and the carotid blood pressure tracings recorded to provide an objective record of the severity of the shock reactions. Cevitamic acid* was injected intravenously in amounts varying from 25 to 100 mg per kilo and after varying intervals of from 15 to 45 minutes an injection of 2 cc per kilo of a 10% solution of a proteose peptone† was made. Definite shock reactions occurred in 10 animals, 2 being rapidly fatal, 5 quite severe, and 3 mild. The distribution of the varying grades of severity of shock was similar with that in a large number of controls.[3] Blood samples were drawn approximately 5 minutes after the peptone injection and the presence of histamine demonstrated in all instances in which the reaction was severe.[3]

It is concluded that the prior administration of Vitamin C to dogs does not protect against peptone shock and that correspondingly it does not prevent the liberation of histamine from the fixed cells of the body into the blood.

9967 P

Vitamin C and Anaphylactic Shock in Dogs.

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Contradictory reports upon the effect of Vitamin C on the anaphylactic reaction of guinea pigs have appeared. Hochwald,[1]

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[3] Dragstedt, C. A., and Mead, F. B., *J. Pharm. and Exp. Ther.*, 1937, **59**, 429.

* We are indebted to Merck and Co. for the Cebione used in these experiments.

† Bacto-Protone-Difco was used in these experiments.

[1] Hochwald, A., *Z. f. d. ges. Exp. Med.*, 1935, **97**, 433.

Lemke,[2] and Solomonica[3] have reported protective effects, while Schwartz and Cisloghi[4] and Van Niekerk[5] have reported negative effects. Giroud and Giroud[6] have reported protective effects in rabbits. To our knowledge no study of the effect of administered Vitamin C on the anaphylactic reaction in the dog has been reported.

Eighteen dogs were sensitized to horse-serum. After incubationary periods of 15 to 20 days they were anesthetized and the blood-pressure reactions recorded to provide an objective record of the degree of shock produced by the intravenous injection of horse-serum. Four dogs were given 250 mg of cevitamic acid* daily by mouth throughout the incubationary period, 5 dogs were given intravenous injections of 200 mg prior to the sensitization, a daily oral dose of 500 mg during the incubationary period and a final intravenous injection of 500 mg before the provocative injection of horse-serum, and 9 dogs were given intravenous injections of 100 mg per kilo during the last 45 minutes before the provocative injection of horse-serum. Sixteen animals showed anaphylactic reactions, of which 4 were rapidly fatal and 9 were quite severe. The distribution of the various grades of severity of shock were similar with that in a large number of controls.[7] Through the courtesy of Professor C. J. Farmer, the concentration of Vitamin C in the blood was determined in some of the animals.

We are thus enabled to say that the blood concentration was elevated from normal levels of approximately 0.20 mg % to around 0.6 mg % in the first group, that in the second group the blood concentration was moderately high at the time of sensitization (2.0 to 4.0 mg %), was maintained at fairly high levels during the incubationary period (around 1.0 mg %) and was considerably elevated (11.0 to 20.0 mg %) at the time of the provocative injection of horse-serum, and that in the third group the blood concentration was above 20 mg % at the time of the provocative injection of horse-serum. It is therefore believed that the amounts of cevitamic acid used were adequate to influence the reactions if such administration has any specific effect.

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[2] Lemke, C. H., *Monatschr. f. Kinderh.*, 1936, **67**, 244.

[3] Solomonica, B., *J. Immunol.*, 1936, **31**, 209.

[4] Schwartz, E., and Cisloghi, F., *Minerva Medica*, 1935, **2**, 522.

[5] Van Niekerk, J., *J. Allergy*, 1937, **8**, 446.

[6] Giroud, P., and Giroud, A., *C. R. Soc. d. Biol.*, 1936, **121**, 1588.

* We are indebted to Merck and Company for the Cebione used in these experiments.

[7] Dragstedt, C. A., Mills, M. A., and Mead, F. B., *J. Pharm. and Exp. Ther.*, 1937, **59**, 359.

Although the number of animals used is not large enough to detect a slight change in response it is evident that the administration of Vitamin C in the manner and amounts here employed has no marked effect either upon the phenomena of sensitization or the subsequent anaphylactic reaction in dogs.

9968

An Antidiuretic Substance Present in the Urine of Dehydrated Rats.

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Gilman and Goodman[1, 2] have reported the discovery of an antidiuretic substance, which they identify as pituitrin, in the urine of animals dehydrated for varying periods of time. These authors have suggested that the effective concentration of a hormone responsible in any part for the reabsorption of water in the renal tubules must increase as the demand for reabsorption becomes greater, as in states of dehydration. It has, further, been reported by Heller[3, 4] that a portion of pituitrin injected into the animal body appears in the urine.

We have repeated a part of the work of Gilman and Goodman, and are able to confirm their statement that an antidiuretic substance can be recovered from the urine of dehydrated rats.

Healthy, adult male rats were deprived of water for periods of 24, 48, and 72 hours. Their urine was collected during 24-hour periods in sufficient 1% acetic acid to keep the samples weakly acid. An attempt was made to keep the samples free from contamination with fecal matter by lining the collecting funnels with glass wool. In most cases, this was successful. Urine so collected was filtered and dialyzed from 5 to 8 hours against running water, through a "viscose" membrane with a wall thickness of 0.0008 inch. It was then concentrated *in vacuo* to approximately 10 cc. When it was

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[1] Gilman, A., and Goodman, L. S., *Science*, 1936, **84**, 24.

[2] Gilman, A., and Goodman, L. S., *J. Physiol.*, 1937, **90**, 113.

[3] Heller, H., and Urban, F. F., *J. Physiol.*, 1935, **85**, 502.

[4] Heller, H., *J. Physiol.*, 1937, **89**, 81.

necessary to hold the urine for any length of time between steps in the processing, it was kept in an ice box.

The assay for antidiuretic activity was performed according to the method of Burn[5] with the exception that the time required for the excretion of one-half the fluid given by gavage was used as an index of diuresis, rather than the time necessary to reach the point of maximum rate of excretion. These 2 points correspond closely. Healthy male rats weighing between 200 and 300 g were used. Each rat was given tap water to the extent of 10% of its body weight by stomach tube. Each urine concentrate was assayed collectively on 4 rats. Each rat was injected with 2.5 cc of the urine concentrate. Urinary output was measured at 15-minute intervals. Interpolation within 15-minute periods was performed when necessary to determine the "mid-excretion point."

Results. (A) Control Group. Fifty control determinations were made. Instead of urine concentrate, 2.5 cc tap water was injected into each rat of an assay group. The time required to reach the "mid-excretion point" was 87 ± 2.8 minutes. This figure corresponds closely with the figure 86 ± 11 minutes reported by Heller[4] as the result of 36 similar control determinations.

(B) Non-dehydrated Group. Six determinations were made on the urine of groups of 12 rats collected during a 24-hour period in which the animals had free access to water. The time required to reach the "mid-excretion point" was 100 ± 5.7 minutes.

(C) Rats Dehydrated 24 Hours. Six determinations were made on the urine of groups of 14 rats collected during a 24-hour period in which the rats received no water. The time required to reach the "mid-excretion point" in this series of tests was 147 ± 19 minutes.

(D) Rats Dehydrated 48 Hours. Nine determinations were made on the urine of groups of 14 rats collected during the last 24 hours of a 48-hour period during which the animals were allowed no water. The "mid-excretion point" in this series was reached after 134 ± 13 minutes.

(E) Rats Dehydrated 72 Hours. Ten determinations were made on the urine of groups of 14 rats collected during the last 24 hours of a 72-hour period of water deprivation. The time required to reach the "mid-excretion point" in this series was 137 ± 9.2 minutes.

The foregoing results are summarized in Table I.

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[5] Burn, J. H., *Quart. J. Pharm.*, 1931, **4**, 517.

TABLE I.

	No. of assays	Mean "mid-excretion time"	Probable error
A. Control group	50	87	± 2.8
B. Non-dehydrated rats	6	100	± 5.7
C. Rats dehydrated 24 hours	6	147	±19.0
D. " " 48 "	9	134	±13.1
E. " " 72 "	10	137	± 9.2

(F) Pitressin Controls. Forty cc human urine from a non-dehydrated subject was added to 20 cc of 1% acetic acid. The solution was exposed on our collecting stand for 48 hours, then filtered, dialyzed and concentrated. Upon assay it produced a "mid-excretion point" at 94 minutes. To a similar quantity of the same urine 50 mille units of pitressin were added, and the same process repeated. This produced a "mid-excretion point" at 181 minutes. Urine to which 200 mille units of pitressin were added, and similarly treated produced a "mid-excretion point" at 280 minutes.

In our series there is no significant difference between the antidiuretic effect of tap water and the urine concentrate of well hydrated rats, for the difference between the means of these groups is 13, a figure less than 3 times the probable error in the assays. There is, however, a significant difference between the control mean and that obtained by the injection of the urine concentrate of dehydrated animals.

It will be noted that in our series the amount of antidiuretic substance excreted reached a fairly uniform level after 24 hours of dehydration, and remained at about this level for the duration of the dehydration period. In this respect, our results differ from those of Gilman and Goodman, who found that the excretion of antidiuretic substance reached a maximum level at the end of 48 hours' dehydration and remained at about this level during periods of dehydration ranging up to 96 hours. The fact that a level of excretion of antidiuretic substance is reached and maintained is in accord with the observation of Heller[4] that within certain ranges, the amount of antidiuretic substance excreted by the kidney during experiments in which the hormone (pituitrin) is added to the blood, is definitely independent of the blood hormone concentration.

Ingram, Ladd and Benbow[6] have recently reported the detection of antidiuretic substance in the urine of dehydrated but not normal cats. Results somewhat contradictory to ours and those of

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[6] Ingram, W. R., Ladd, L., and Benbow, J. T., *Proc. Am. Physiol. Soc.*, April, 1938, p. 107.

Gilman and Goodman have recently been reported in abstract by Walker.[7]

Conclusion. An antidiuretic substance can be obtained from the urine of dehydrated rats. This substance is not present in significant quantities in the urine of non-dehydrated rats. Its action upon water diuresis is similar to that of pitressin, though we cannot say that it is pitressin.

9969

Effect of Choline on Atherosclerosis in the Rabbit Aorta.*

C. A. BAUMANN AND H. P. RUSCH. (Introduced by W. J. Meek.)

From the Department of Physiology, University of Wisconsin.

The cholesterol content of rat liver is increased by the feeding of cholesterol,[1, 2] and its accumulation is accompanied by the deposition of large amounts of neutral fat. The increased cholesterol is chiefly in ester form.[3] The fatty livers produced by feeding 1% of cholesterol can be prevented by feeding large amounts of choline.[2] In the rabbit, cholesterol feeding results in the deposition of cholesterol in the aorta in the form of atheromatous plaques.[4-8] Atherosclerosis in rabbits has been prevented by feeding a pancreas extract.[9] The antagonism exhibited between choline and cholesterol deposition in the rat led us to investigate the effect of choline on the formation of plaques in the rabbit aorta.

Twelve female rabbits, 1.3 to 3.0 kg in weight, averaging 2.14 kg were divided into 3 groups. The first group received Purina

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[7] Walker, A. M., *Proc. Am. Physiol. Soc.*, April, 1938, p. 210.

* Supported in part by the Jonathan Bowman Cancer Fund, and the Wisconsin Alumni Research Foundation.

[1] Chanutin, A., and Ludewig, S., *J. Biol. Chem.*, 1932, **102**, 57.

[2] Best, C. H., Channon, H. J., and Ridout, J. H., *J. Physiol.*, 1934, **81**, 409.

[3] Okey, R., PROC. SOC. EXP. BIOL. AND MED., 1933, **30**, 1003.

[4] Cowdry, E. V., *Arteriosclerosis*, Macmillan & Co., 1933.

[5] Rosenthal, S. R., *Arch. Path.*, 1934, **18**, 473.

[6] Menne, F. R., Beeman, J. A. P., and Labby, D. H., *Arch. Path.*, 1937, **24**, 612.

[7] Turner, K. B., and Bidwell, E. H., PROC. SOC. EXP. BIOL. AND MED., 1937, **35**, 656; 1935, **62**, 721.

[8] Aylward, F. X., and Stott, Wm., *Biochem. J.*, 1937, **31**, 2055.

[9] Huber, M. J., Brown, G. O., and Casey, A. E., PROC. SOC. EXP. BIOL. AND MED., 1937, **37**, 441.

rabbit chow *ad libitum;* the second, rabbit chow plus 0.43% cholesterol and 5% hydrogenated cottonseed oil (Primex); the third, rabbit chow plus cholesterol and 715 mg of choline hydrochloride (Eastman) per liter of drinking water. The animals were kept in groups and daily water consumption of each group was recorded. This gave the average intake of choline for each rabbit. The cholesterol was dissolved in heated primex and poured over the rabbit chow and thoroughly mixed. The blood was analyzed periodically for cholesterol. At the end of 4 months, the animals were killed, the aorti examined both grossly and histologically, and analyzed for free and ester cholesterol. Determinations of fat and cholesterol were also made on liver, kidney, spleen, and lung tissue. The cholesterol was determined by a modification of the Schoenheimer-Sperry method.[10] In a second series, the cholesterol content of the diet was reduced to 0.22%, and the choline hydrochloride given in concentrations of 0.5 or 1.0 g per liter of drinking water.

All diets were readily consumed and the animals gained in weight from an average of 2.14 kg to 3.44 kg in Series I; from 1.53 kg to 3.37 kg in Series II. Two deaths from unknown causes occurred during the second experiment, one on the cholesterol diet, the other on cholesterol plus the higher level of choline.

A marked increase in blood cholesterol was observed on all the levels of cholesterol fed, irrespective of the choline intake. In our first series the total blood cholesterol rose from 1.13 to 6.21 mg/cc. In our second series, the increase was from 1.13 to 9.9 mg/cc. The increases occurred in both the free cholesterol and the ester fractions, the greatest increases being in the latter. The maximum value for blood cholesterol was attained in from 6 weeks to 2 months on the higher level, 0.43% of cholesterol, whereas on the lower level (0.22%), the maximum was reached between the third and fourth month. However, the maximum values of blood cholesterol attained were actually higher on the lower level of intake (Table I). In neither series did choline exert any detectable effect on the cholesterol content of the blood.

The results of tissue analyses from the high cholesterol series are given in Table II. Fatty livers resulted from feeding 0.44% cholesterol, the values rising from a fat content of 3.89% of the fresh weight on the control diet to 9.04% on cholesterol and 13.7% on cholesterol plus choline. The level of choline employed, therefore, failed to reduce the deposition of liver fat. The cholesterol content of the control livers was 10.8 mg per gram. Cholesterol

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[10] Schoenheimer, R., and Sperry, W., *J. Biol. Chem.*, 1934, **106**, 745.

TABLE I.
Cholesterol in Rabbit Blood, mg/cc whole blood.

	Series I			Series II			
Time	Control	.43% cholesterol	.43% cholesterol + 121 mg choline daily	Control	.22% cholesterol	.22% cholesterol + 108 mg choline	.22% cholesterol + 216 mg choline
0	1.00	1.13	1.09	1.27	1.22	1.13	1.21
2 wk	1.18	1.35	1.87				
1 mo.	1.24	4.65	6.17	1.28	3.98	3.69	3.33
6 wk	1.31	6.21	5.58				
2 mo	1.80	4.92	6.18				
3 ”	.94	4.96	4.99	1.61	6.1	9.9	7.36
4 ”				1.35	8.1	7.24	8.92

TABLE II.
Effect of Cholesterol and Choline on Rabbit Tissues.

	No. of animals	Cholest. in diet %	Avg cholest. consumed daily mg	Choline conc. g/L	Avg daily consump. of added choline mg	Avg daily consump. of “Choline” (ration) mg	Avg total choline consumed daily mg	Liver fat %	Liver cholest. range mg/g	Liver cholest. avg mg/g	Aorta cholest. range mg/g	Aorta cholest. avg mg/g
Series I												
Controls	4	—	—	—	—	82	82	3.89	7.0-18.5	10.8	5.7-13.7	8.6
Cholesterol	4	.43	460	—	—	82	82	9.04	12-25	19.2	23-41	30.7
Cholesterol + choline	3	.43	460	.715	121	82	203	13.7	17-29	24.4	14-54	31.9
Series II												
Controls	2	—	—	—	—	82	82	4.0	11.8-13	12.4	5.3-6.2	5.8
Cholesterol	3	.22	240	—	—	82	82	6.9	10.7-11.8	11.2	21-48	31.6
Cholesterol + low choline	4	.22	240	.50	108	82	190	7.0	8.4-16.6	11.6	26-37	30.7
Cholesterol + high choline	3	.22	240	1.00	216	82	298	6.4	9.2-14.8	12.0	11.8-30	22.6

feeding raised this level to 19.2 mg per gram, and cholesterol plus choline, to 24.4 mg per gram. Dietary choline was therefore without effect in reducing liver cholesterol.

No fatty livers were observed on the lower levels of cholesterol fed (Table II). The liver cholesterol was remarkably constant on all these diets; it varied from 11.2 to 12.4 mg per gram; the highest value, incidentally, was obtained on the control diet. Choline, therefore, was without effect on either liver fat or liver cholesterol.

The aorta, on the contrary, like the blood, was profoundly affected by the ingestion of 0.22% cholesterol (Table II). Characteristic atheromatous plaques were observed in the aorti of all animals receiving cholesterol, regardless of the amount of choline ingested. The lesions were similar to those described in detail by others,[4-8] and while considerable variation was observed between individuals, there were no obvious differences between the various groups. This conclusion is supported by the results of chemical analyses. The cholesterol content of the normal aorta was 5.8 mg per gram. The aortas of cholesterol-fed rabbits contained 31.6 mg per gram. The administration of choline failed to affect the cholesterol content of the aorta in 6 out of 7 rabbits. The aorta of the seventh rabbit contained only 11.8 mg cholesterol per gram, and both gross and histological examination showed a decreased number of lesions. The blood of this rabbit, however, like that of the others in the group, contained large amounts of cholesterol. It is doubtful, therefore, whether the decreased number of atheromatous lesions should be attributed to the action of choline. The animal may have come from a different strain than the others of the series, since this was the only one with yellow body fat.[†]

On the higher level of cholesterol fed, (0.43%) atheromatous lesions, accompanied by a high cholesterol content, 14.6 to 54.6 mg cholesterol per gram of aorta, were observed in all animals (Table II) irrespective of choline intake. The cholesterol content of rabbit kidney was relatively constant on all diets, and the variations in the cholesterol content of the spleen had little relation to the diets employed.

Summary. Fatty livers, with some increase in liver cholesterol, were produced by feeding rabbits a diet containing 0.43% cholesterol; 0.22% cholesterol produced no changes in liver fat. Both levels of cholesterol produced marked atheromatous lesions, and a striking increase in the cholesterol content of the aorta and blood. Three hundred mg of choline hydrochloride daily failed to counteract the effect of cholesterol in the blood, liver or aorta.

[†] A recent report (Steiner, A., PROC. SOC. EXP. BIOL. AND MED., 1938, **38**, 231) states that 500 or 750 mg choline hydrochloride daily failed to prevent atheromatous lesions in rabbits induced by a weekly intake of 3 g of cholesterol.

9970 P

Use of Ascitic Fluid in the Treatment of Primary Shock.

Roger M. Choisser and Elizabeth M. Ramsey. (Introduced by Earl B. McKinley.)

From the Department of Pathology, School of Medicine, George Washington University, Washington, D. C.

It has been abundantly demonstrated that the symptoms of primary shock can be relieved by prompt administration of fluid to replace that diverted from the general circulation. Blood is the ideal fluid for this purpose since its protein content prevents too rapid elimination by the kidneys. Of the blood, the serum alone is vital to this action. However, transfusion cannot be performed without time-consuming preliminaries. The ideal fluid for emergency use would, therefore, be some stable, universally compatible protein solution which might be kept on hand at all times. A sterile body transudate would present several advantageous features, in particular the nature of its protein content which is identical with that in blood serum. Ascitic fluid, among the transudates, would be especially desirable since it is elaborated in non-infectious conditions and is available in most clinics in large amounts.

A study of ascitic fluid has been carried out in this laboratory to determine its suitability for this use. Nineteen specimens of ascitic fluid were used, the majority of which were obtained from cases of advanced portal cirrhosis of the liver. All specimens were filtered under aseptic conditions, cultured for sterility and their respective agglutinins determined. Protein and pH determinations were carried out in the usual manner. All fluids were found to be not only sterile but strongly bacteriostatic. The pH was uniformly in excess of 8.65. This was reduced to 7.5 with N 10 hydrochloric acid prior to administration. The protein content ranged from 2.1 to 4.2 mg %. It was found impractical to remove the agglutinins by the classic globulin precipitation method, as dialysis to remove the ammonium sulphate produced too great a dilution. Many of the agglutinins were amenable to destruction by heat—especially the

B agglutinins, others were only temporarily suppressed by this treatment.

The effect of intravenous administration of ascitic fluid was studied in 10 dogs weighing 10 to 15 kg. A basal nembutal anesthesia was employed and, after stabilization of the blood pressure, shock was produced by withdrawal of blood, by trauma or by a combination of hemorrhage and trauma. Twenty minutes following a shock-level drop in blood pressure, an amount of compatible ascitic fluid (1500 cc) sufficient to contain a concentration of protein equivalent to that in 1000 cc of whole blood was slowly administered intravenously. An immediate rise in blood pressure to 90% of the original reading was effected. This was maintained for 2½ hours, at the end of which time the experiment was terminated. Repetition of this experiment, using the animal's own citrated blood in the full amount removed, returned the pressure to the original volume. The use of a 0.9% solution of sodium chloride effected a 68% return which was maintained for only 15 minutes. Following this there was a gradual drop in pressure to the previous shock level associated with diuresis.

The favorable results with ascitic fluid were obtained only when a fluid naturally devoid of agglutinins was used. The use of fluids containing naturally incompatible agglutinins or fluids in which incompatible agglutinins had been temporarily suppressed by heat resulted in an initial restorative effect like that described, followed after several hours by a rapid fall in pressure and death of the animal. Subsequent necropsy of these animals revealed multiple infarctions of lungs and brain. Reinvestigation of the "agglutination suppression" phenomenon showed it to be effective only for human cells. The agglutinating activity of the fluid was retained with respect to dog cells.

Summary. Sterile ascitic fluid of high protein content, free from agglutinins, is of value in combatting artificially produced primary shock in dogs. The effect is immediate and prolonged. The percentage return of blood pressure closely approximates that obtained when whole blood is used.

9971

Effects of Insulin Shock on Behavior and Conditioned Reflex Action in the Well Trained Sheep.*

J. A. Rose, A. Tainton-Pottberg and O. D. Anderson. (Introduced by H. S. Liddell.)

From the Department of Physiology, Cornell University Medical College, Ithaca, N. Y.

Insulin shock has radically, and perhaps permanently, altered the behavior of a sheep whose conditioned reflexes and general behavior have been studied for 7 years. During the year preceding the insulin experiment the conditioned motor reflex had gradually failed (Fig. 1-A) although the sheep remained quiet and alert during the 20-minute test period. The sheep was started on 10 units of insulin and the daily dose was increased by 10 to 15 units until coma and convulsions occurred. First signs of coma were observed at 160 units but dosage was carried to 280 units in an effort to secure typical shock phenomena. Convulsions were induced on 7 successive days.

No discrepancies in the results as given below for a typical day were observed. Insulin is given to the fasting sheep at 9 A. M.; in 5 hours it is standing with head drooping, lips twitching, and must be carried into the testing laboratory. It is placed upon the observation platform with great difficulty. When in the testing frame the sheep is induced to stand but soon slowly sinks. After a few minutes it rises and stands for a few minutes, then sinks again. When standing, the head gradually droops, the eyelids close; then suddenly the head is raised and the eyes are opened. This sequence is repeated many times throughout the first part of the observation period.

At this time the heart rate is normal or below normal (80-60); breathing is labored and noisy. The animal is now tested. It not only shows no conditioned motor (defensive) reflex but fails to respond to any outside stimulus. In an effort to show what is happening to the animal at all times, the conditioning test (sound of buzzer for 10 seconds followed by shock to the left foreleg) is repeated at the usual 5-minute intervals in the coma, the recovery period, and for some time after. After about an hour of fairly

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* The expenses of this investigation were defrayed in part by a grant from the Josiah Macy, Jr., Foundation.

severe convulsions glucose is administered by mouth. In about 30-40 minutes the animal becomes more alert. It stands, eyes open, head no longer drooping, and repeated head movements are seen. At this time the sheep shows evidence of beginning hyperexcitability.

In the intervals between conditioning tests the sheep which for-

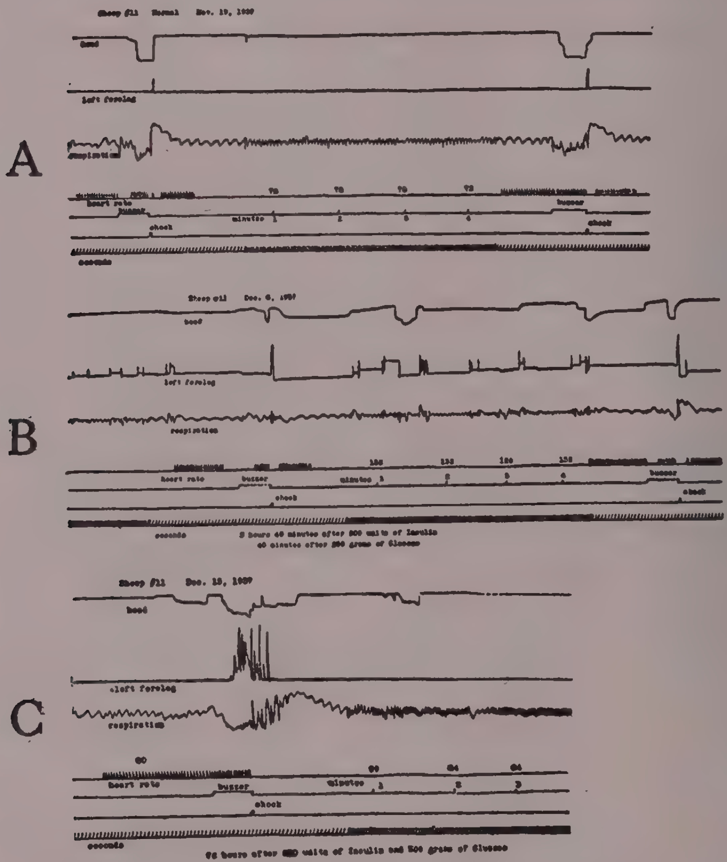

FIG. 1.

In each record, reading from top to bottom: head movement, foreleg movement, respiration, heart rate, signal, shock, time in seconds.

A. A graphic record of the behavior of a normal sheep. Note the absence of the conditioned foreleg movement to the buzzer. The animal is perfectly quiet in the interval between the two buzzer tests.

B. A record of the behavioral reactions of the same animal a short time after insulin shock. Note that the animal shows frequent restless movements in the interval between the two conditioned stimuli. Contrast this record with that of A.

C. A record of the behavior of this animal 3 days after the series of insulin shocks. Note the extreme vigor of the conditioned reflex elicited by the buzzer (conditioned) stimulation.

merly stood without movement now exhibits spontaneous flexion movements of the reaction leg (left foreleg). These occur with great frequency and are accompanied by an increase in respiration and pulse rate as observed in experimental neurosis (Fig. 1-B). The defensive reaction of the limb to shock (in abeyance during coma) shortly manifests itself.

About an hour after the glucose administration, signs of overactivity are still quite evident. The *conditioned reflex which has been almost entirely absent for one year now reappears with abnormal vigor.* The *interval leg movements* decrease in frequency as the tests continue and the amplitude of the conditioned movements (*i. e.,* during buzzer signal) increases as they decrease (Fig. 1-C). At about 5 P. M. the sheep is returned to its pen and fed. Pulse and respiration are approximately normal.

In the recovery period from each of the 7 insulin shocks the movements of the reaction limb (left foreleg) in the rest intervals between signals appeared for the first time in the history of this animal and occurred at times with a frequency as high as 6 per minute. Such movements have been observed only in animals exhibiting experimental neurosis. On each occasion the number of such movements gradually decreased and as this occurred the conditioned reflex reappeared. The latter at times attained a value as high as 420 mm as determined by Fick's work adder. Such a conditioned reaction was by far the most vigorous one the sheep had ever given. In the tests made during the period from the last insulin dose on December 12th to the present, March 5th (2½ months) the conditioned responses showed an average magnitude of 185 mm which notably exceeds the average figure for 6 years preceding the experiment (150 mm) and is more than 4 times as great as during the immediately preceding year (39 mm). At present the interval leg movements appear but rarely.

9972

Influence of Progesterone on Experimental Tuberculosis in Male Guinea Pigs.

Meridian R. Greene and Herbert R. Morgan. (Introduced by T. D. Beckwith.)

From the Department of Bacteriology, University of California, Los Angeles.

As the condition of tuberculous patients appears to improve during the early months of pregnancy, we were interested in noting what influence the hormone, progesterone, might have on experimental tuberculosis.

Steinbach and Klein[1] found that pregnant mare serum, Antuitrin-S and Follutein to a less degree, retarded tuberculosis in rabbits and guinea pigs. Since the latter two agents stimulate the ovaries to luteinization, it seemed not unlikely that the effect on tuberculosis might be due to a hormone from the corpus luteum, which develops and persists during pregnancy.

Male guinea pigs were used to eliminate fluctuations in secretion of hormones from the ovaries that would occur in the female. Animals used had an initial range of weights from 370-470 g and were negative to tuberculin. Twenty were inoculated subcutaneously with 1 mg of the C3 strain of bovine tubercle bacilli. On the same day, ½ unit of progesterone* was given to each of 10 of these animals and to a non-infected one by the subcutaneous route. The dose was administered 3 times weekly for 6 weeks. One non-infected animal which was not given progesterone was included in the series. The hormone preparation was an oil solution and contained one rabbit unit per cc as defined by Parke, Davis & Co. The original culture of tubercle bacilli was obtained from the laboratories of the New York City Department of Health. The culture used had been grown for 3 weeks on Petroff's medium and the suspension was made in saline. At autopsy, the animals were scored for tuberculosis by the method of Petroff and Steenken.[2] Tissues from 8 infected animals, of which 4 had received progesterone, and tissues from the 2 non-infected animals were fixed in Zenker's solution. Alternate paraffin sections were stained by the hematoxylin and eosin and Ziehl-Neelsen methods.

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[1] Steinbach, M. M., and Klein, S. J., *J. Exp. Med.*, 1937, **65**, 205.

* We appreciate the courtesy of Parke, Davis & Co. in supplying us with Lipo-Lutin.

[2] Petroff, S. A., and Steenken, W., *J. Immunol.*, 1930, **19**, 79.

TABLE I.
Macroscopic Tuberculosis in Animals Given Progesterone and Control Animals.

Animal No.	Lung	Liver	Spleen	Kidney	Lymph nodes Inguinal	Retroper-itoneal	Tracheo-bronchial	Hepatic	Mesenteric	TB score	Duration of experiment
Experimental											
2-1*	3+	2+	2+	0	M‡	0	3	2	0	3+	47
2-2	2+	3+	2+	0	M‡	1†	2†	1†	1†	3+	46
2-3■	2+	4§	4+	0	M†	2†	2†	1†	0	4+	47
3-1	1+	2+	4+	0	M‡	2†	3†	1	0	3+	46
3-2	3+	2+	2+	0	M‡	2†	4†	2	1†	3+	46
3-3	2+	3+	3+	0	M‡	3†	3	1	1	3+	38
4-1*	1+	1+	3+	0	M†	3†	4	3	1	3+	45
4-2	1+	1+	1+	0	M‡	3†	2	0	0	2+	24
4-3■	3+	3+	2+	0	M‡	3†	3†	1	2	3+	45
5-1	2+	4§	1+	1+	M‡	3†	3	3	0	4+	46
Control											
5-2	1+	3+	2+	1+	M†	4†	3†	4	0	3+	46
6-1*	4+	2+	1+	0	M‡	5†	2†	1†	0	3+	47
6-2*	1+	4§	3+	0	M‡	2†	2†	1†	0	4+	47
6-3	1+	3+	2+	0	M‡	2†	2†	3†	0	3+	46
7-1	2+	3+	2+	0	M‡	1†	2	0	0	3+	38
7-2	2+	3+	2+	1+	M‡	2†	5†	2†	0	3+	46
7-3	1+	4§	2§	1+	M‡	2†	3†	1	0	4+	46
8-1	1+	1+	1+	0	M†	2†	3	0	0	2+	24
8-2*	2+	3+	3+	0	M†	2†	2†	1	0	3+	45
8-3*	3+	3+	2+	0	M†	3†	5†	1	1	3+	45

*Sections taken.
†Caseous.
M‡ Much enlarged, caseous, and ulcerated.
§Infarct.

Four weeks after inoculation, the animals receiving hormone weighed, on an average, 80 g less than the controls. At 2 weeks, tuberculin reactions were 3+ in both groups. All animals had inguinal lymph nodes which were much enlarged. At 4 weeks, the reactions were less intense and several animals did not respond to tuberculin. The variations were common to both groups. Most animals had ulcerations at the site of inoculation in the inguinal region. Non-infected guinea pigs were negative to tuberculin.

One animal from each group was killed on the 24th day and on the 38th day and the remaining guinea pigs were sacrificed 45-48 days after inoculation. Each of the latter animals receiving hormone had been given a total of 9 units.

Individual scores for macroscopic tuberculosis ranged from 2+ to 4+ (Table I). The average score for the tuberculous animals which received progesterone was 3.1. That for animals not given hormone was also 3.1. Variations in type and extent of lesions in various organs were common to both groups.

Sections were prepared from the lung, liver, spleen and some of the lymph nodes. These showed the presence of tubercle bacilli. Involved areas consisted of single and confluent miliary tubercles made up of epithelioid and giant cells with a peripheral zone of lymphocytes. Central necrosis was not common. Extensive caseation was rare except in the lymph nodes. Sections from the kidney and testis revealed no involvement with tuberculosis.

The dose of tubercle bacilli was large but the degree and extent of involvement was not maximum in control animals. A decided retardation of the tuberculous process by progesterone would probably have manifested itself. However, it might be that the hormone would show some effect in female animals.

Development of a tuberculous infection in male guinea pigs was not retarded by small doses of progesterone nor was there an altered skin sensitivity to tuberculin.

9973 P

Protective Action of Typhoid Phage on Experimental Typhoid Infection in Mice.

Roy T. Fisk. (Introduced by John F. Kessel.)

From the Department of Bacteriology, School of Medicine, University of Southern California, and the Los Angeles County Hospital, Los Angeles.

Asheshov, Wilson and Topley[1] have reported on the protective effect in mice of typhoid bacteriophage given separately and by routes different from those used in infecting the animals. The preliminary studies here reported confirm the findings of the above investigators and enlarge upon certain aspects of the problem.

A recently isolated strain of *Eberthella typhi* obtained by blood culture was employed. It forms smooth colonies, is motile, produces typical sugar fermentation reactions and agglutinates specifically with anti-typhoid serum. Organisms from 18- to 24-hour-old cultures grown on veal infusion agar were suspended in mucin* according to the methods of Rake[2] and Miller,[3] and mice were infected by the peritoneal route, all inoculations being made with a quantity of 1 cc of the mucin-organism mixtures.

The phage was originally isolated from sewage and is a crude phage which produces several different types of plaques. Filtrates which contained approximately 3.3×10^8 phage particles per cc were used for mouse injection.

Typhoid infection in mice was acute. Blood stream invasion with resulting bacteremia usually occurred within 1 hour following intraperitoneal inoculation and all of the fatally infected control mice died within 24 hours. The minimal fatal dose of the culture varied in 7 virulence tests from 1000 to 10 bacilli, thus the quantity of organisms used in the phage experiments represented from 1000 to 100,000 fatal doses.

The protective effect of phage was tested by various ways in 7 experiments, the results of which may be summarized as follows:

(1) Phage injected immediately before the intraperitoneal inoculation of 1×10^6 typhoid bacilli protected all of 32 mice when injected intravenously and all of 19 mice when administered by the subcutaneous method. Intravenously injected phage also protected

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[1] Asheshov, I. N., Wilson, J., Topley, W. W. C., *Lancet*, 1937, **232**, 5919, 319.

* Granular mucin manufactured by Wilson Laboratories, Chicago, Ill.

[2] Rake, G., Proc. Soc. Exp. Biol. and Med., 1935, **32**, 1523.

[3] Miller, C. P., *J. Inf. Dis.*, 1936, **58**, 263.

against $1x10^7$ and $1x10^8$ bacilli, but failed to protect against $5x10^8$ organisms which may have been due in part to the primary toxicity of such a large number of organisms.

(2) The amount of phage required to protect mice against $1x10^6$ bacilli was found to be quite small since $1x10^{-4}$ cc afforded complete protection to 10 mice. Smaller amounts of phage showed a diminishing effect.

(3) In other experiments it was found that mice remain resistant to infection for as long as 24 hours following intravenous phage injection. The persistence of resistance following other methods of phage injection or for longer periods after intravenous injection has not yet been determined.

(4) The therapeutic effect of phage was tested by injecting phage intravenously at various periods of infection up to 4 hours after typhoid inoculation. Blood cultures taken in every instance before phage injection showed bacteremia to be present in 23 of the 29 mice of this experiment. The fact that all of these mice survived even when phage injection was delayed for 4 hours shows that phage exerts a protective effect even after generalized infection has taken place.

(5) Control mice were included in each experiment. Among the untreated controls 44 of 48 mice died and in another group 19 out of 20 mice treated with phage heated to 70°C for 50 minutes developed a fatal infection. Thus among the 68 control mice there was a mortality of 92.6%.

It is apparent from the results obtained in this study that bacteriophage exerts a protective action in experimental *Eberthella typhi* infection in mice. Asheshov, Wilson and Topley[1] who worked with the "Rejuvenated Rawlings" strain of *E. typhi* and both pure and mixed phages also demonstrated the protective effect of phage. They record, however, that 23 of 30 mice died when phage injection was delayed for 4 hours. The use of mucin in the present study made possible the inoculation of smaller numbers of bacilli which perhaps explains the more striking curative results obtained.

9974 P

Function of the Giant Fibers of the Central Nervous System of the Crayfish.

C. A. G. Wiersma.

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Johnson[1] has made studies about the course, the connections and the function of the giant fibers of the central nervous system of a few crustaceans, especially of Cambarus. Without actually isolating these fibers, he came to the conclusion that both sets of giants, described as the median and the lateral giants, have the function of flipping the tail. The median giants seem to connect with the motor fibers of each abdominal segment by a very simple contact mechanism; the connection of the lateral giants with the motor fibers is not as well known.

With the same method as used in the studies on the peripheral nerve-muscle system of crustaceans[2] it was possible to isolate in the oesophageal commissures these 2 sets of giant fibers of *Cambarus clarkii* and to stimulate them separately. The median giant fibers are considerably thicker than the lateral ones, the former have a diameter which varies between 200μ and 250μ, whereas the latter measure 60μ to 90μ in the animals used.

It was found that single induction shocks applied to the unprepared oesophageal commissure or to any of the 4 giants caused a twitch-like contraction of the flexor muscles of the tail, a stretching of the legs and an inward movement of the antenna. The other fibers in the oesophageal commissures do not give a noticeable reaction to single shocks (with faradic stimulation various effects may be obtained from them). The tail contractions were registered either isotonically with load or isometrically and it was found that the effect of single shocks applied to any of the 4 fibers did not vary with the strength of the stimulus within wide limits. The threshold contraction is of a considerable strength and only with very strong shocks is a higher contraction obtained, which is due either to stimulation of the other giant fiber in the same commissure by current escape, or to a repeated discharge in the stimulated fiber. There can thus be no doubt about the validity of the all-or-none relation of this system, which is the more convincing as the series of connections which each giant makes in the row of ganglia would be an ideal opportunity for decremental conduction to show, as the number of ganglia reached would be then proportional to the strength of the stimulus.

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[1] Johnson, G. E., *J. Comp. Neurol.*, 1924, **36**, 323; 1926, **42**, 19.

[2] Harreveld, A. van, and Wiersma, C. A. G., *J. Physiol.*, 1936, **88**, 78.

The question whether these fibers are really intracentral fibers and thus connected with the peripheral neuron by a synapse must be answered in the affirmative. It is possible to stimulate separately the 3 peripheral nerve bundles which leave each ganglion on both sides. Single shocks of moderate strength never result in a real twitch of the tail. Only on stimulation of the third root is some flexion obtained, but this contraction is localized to the segment innervated by this root. After cutting a third root, stimulation of the peripheral part has an effect similar to that obtained before the cutting, whereas stimulation of the central stump does not result in a visible effect unless the stimulus is made so strong that a stimulation of the giant fibers in the chain is possible by current escape.

Experiments on the summation of 2 single induction shocks given to 2 different giants were carried out in 3 combinations. For the 2 median giant fibers it was found that no summation occurred when they were stimulated simultaneously. When either one was stimulated before the other with intervals up to about 1.3σ there was no summation. With slightly longer intervals there was some summation, which became maximal at an interval of about 1.7σ, and declined slowly with increasingly longer intervals. For the 2 lateral fibers the maximum interval between stimuli during which no summation occurs is about 4.5σ and the first summated contraction is usually also maximal. When a median and a lateral giant are combined, the result is less clear than in the foregoing cases. Often some summation is obtained at all intervals. It should be remarked that the contractions caused by a median and a lateral giant are often not quite equal in height; in a fresh preparation the median usually causes a somewhat stronger contraction than the lateral, but this varies in different animals and in the course of one experiment. There is usually a pronounced summation when these 2 fibers are stimulated simultaneously. When the median giant is stimulated before the lateral one, summation occurs at all intervals at which the 2 contractions fuse. When the lateral giant is stimulated first a range of intervals occurs giving much less or no summation, this range is from about 0.5σ to 3σ; longer intervals again give summation.

These experiments show that a "refractory period" is obtained by stimulation of different fibers, and from this it must be concluded that all 4 giant fibers govern the same peripheral nerve-muscle systems in the tail.

9975

Protoplasmic Potentials in Halicystis. VI. Rôle of Ammonia in Potential Reversal by Perfusion.*

L. R. Blinks.

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Earlier papers[1] have described the reversal of the normally positive potential difference across the protoplasm of impaled cells of *Halicystis osterhoutii* (Bermuda) both by exposure to ammonia (or other weak bases) and by perfusion of the vacuole with more alkaline sap or sea water. The author was then inclined to explain the former effect by the latter, since penetration of ammonia raised the vacuolar pH. One difficulty of this interpretation was that the Californian species, *H. ovalis*, did not show such reversal on perfusion with sea water at pH 8.0 or higher, although reversing well on exposure to ammonia. This difference between the species now seems reconciled by referring the effects in both cases to the penetration of ammonia into the protoplasm. This is shown in *H. ovalis* by introducing into the perfusing fluid a trace of NH_4Cl; at pH 5.0, the normal acidity of the sap, no effect is observed, but if sea water now is perfused at pH 8.0, reversal of potential often results. Then as perfusion is continued, and removes the added ammonia, recovery of positive P.D. may result, even at pH 8.0.

Reëxamination of *H. osterhoutii* in Bermuda shows similar results. Very clear, pale cells, with no evidence of recent reproduction, also do not show reversal of P.D. when perfused with sea water at pH 8.0. This behavior was sometimes found in earlier work, but was then ascribed to imperfect perfusion; such is now definitely not the case, since rapid flow, good mixing in the vacuole, (as shown by tracer dyes), and long continued perfusion were carefully maintained. Certain other cells at first may reverse on such treatment, but later recover positive P.D. on continued perfusion. In both of these cases, addition of a trace of NH_4Cl to the perfusing fluid promptly causes reversal, which can be counteracted by lowering the pH to 6.0 or less:—the effect originally ascribed to pH alone. In such a state also, the P.D. responds to light, in a manner analo-

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* Aided by a grant from the Rockefeller Foundation.

† Contribution from the Bermuda Biological Station for Research, Inc.

[1] Blinks, L. R., *J. Gen. Physiol.*, 1933, **17**, 109; 1935, **18**, 409. The methods employed are described in these papers.

gous to that produced when ammonia is externally applied. This effect, which will be described elsewhere, seems wholly attributable to the pH changes accompanying photosynthesis, acting upon the ammonia dissociation. In the absence of ammonia, the effects of light are much smaller.

Examination showed, however, many cells of *H. osterhoutii* like those originally reported, which display continued reversal of P.D. during perfusion of sea water for long periods. These are generally dark green in color, and have dark masses, the remains of unliberated gametes from previous reproductive periods, floating in the vacuole. Ammonia can be detected in the vacuolar sap of such cells up to 0.005 M or higher by Nessler test; it is possibly derived from the breakdown of the gametes. Obviously considerable dilution of the sap must be necessary to reduce this value below threshold concentrations for reversal, which in this species occurs on external application of 0.0005 to 0.001 M NH_4Cl at pH 8.0. Since perfusion is less certain in these very cases, due to repeated clogging of the outlet capillary by the floating masses, incomplete elimination of the "native" ammonia appears to be the explanation of the earlier results; thus an imperfect perfusion results in reversal, rather than in failure to reverse.

A corrected interpretation for both species is now suggested: Reversal of P.D. is not produced by high pH alone, either in sea water or vacuole; *i. e.,* strong bases are not effective in the physiologically tolerated pH range. But if a weak base, such as ammonia (or amines) is present, either externally or in the vacuole, then raising the pH at that place causes the P.D. to fall or to reverse. This is doubtless due to the penetration of undissociated weak base into the protoplasm, with an expected rise in the protoplasmic pH. How this internal acidity change operates upon the potential mechanism is not known, but it may be upon the charges either of organic ions responsible for the potential, or of surfaces necessary for its manifestation.

9976

Effect of Vitamin E Deficient Diet Upon Skeletal Muscle.

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It has been shown by Evans and Burr[1] that muscular weakness, paralysis, and death might occur in young rats which suckled mothers receiving a diet deficient in vitamin E. Symptoms became obvious when the animals were about 3 weeks of age. The muscles of animals so afflicted show marked histologic changes (Olcott[2]). However, all of the animals are not affected in this way. Many survive and live to maturity even though continued on a vitamin E deficient diet after weaning.

The object of these experiments was to study the functional capacity of the gastrocnemii muscles of adult rats which had been reared on a vitamin E-deficient diet. Through the coöperation of the Biochemistry Department such rats were made available to us for study. The prospective mothers were given a vitamin E-free diet (Olcott[2]) with just enough wheat germ oil to produce almost continuous fertility. Those of the young which survived to the 28th day were weaned and placed on the vitamin E-deficient diet.[2] A part of them which received a wheat germ supplement served as the controls.

The animals were continued on this diet to an age of from 4 to 6 months. At that time the maximum isometric tension developed by the intact gastrocnemius in response to condensor discharges, at the rate of 50 per second, was determined. In addition the creatine, chloride and water concentrations of the gastrocnemius were determined. Some of the muscles were reserved for histological examination. In certain instances the muscles were tested for sensitivity to remote acetylcholine injection.

The results of the tension experiments and chemical analysis are summarized in Table I. The tension developed by the muscles of the experimental males is definitely less than that found for the control animals whether the muscles were stimulated directly or through the peripheral motor nerve. Furthermore, the tension developed by the muscles of the animals on the vitamin E-free diet in response to nerve stimulation was considerably below the tension developed upon direct stimulation. Thus it appears that the vitamin E-deficient diet has resulted in a failure of the muscle to exhibit normal contractile power. It may be noted here that the tensions developed by the muscles of animals receiving the vitamin E-free diet plus wheat germ oil are of precisely the same order as for rats of a similar age reared on our stock diet.

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[1] **Evans, H. M., and Burr, G. O.,** ***J. Biol. Chem.*****, 1928, 76, 273.**

[2] **Olcott, H. S.,** ***J. Nutrition,*** **1938, 15, 221.**

TABLE I.

Sex Diet	♂ E-free	♂ E-free + wheat germ oil	♀ E-free	♀ E-free + wheat germ oil
No. animals	12	8	14	9
Body wt. (g)	235	307	188	206
Muscle tension, g per g muscle				
(a) Nerve stim.	802	1680	1264	1790
(b) Muscle stim.	1188	1773	1412	1774
$\frac{\text{Muscle wt.}}{\text{Body wt.}} \times 100$	0.58	0.53	0.58	0.60
Creatine, mg/100 g muscle	368	451	442	477
% water in muscle	76.6	75.4	—	—
Cl in mg/100 g muscle	71.0	45.0	—	—

The changes in creatine and chloride concentrations are also indicative of muscle dystrophy. The constancy of the muscle weight to body weight ratio in the experimental and control series indicates that there was no gross atrophy.

Histologic examination* showed a focal hyaline necrosis in only a few fibers and an occasional instance of proliferation of sarcolemma nuclei. This alteration in contractile ability of the muscles in the absence of characteristic histologic change is reminiscent of certain types of myopathy in humans where the clinician is able to elicit signs of impaired muscle function while very little pathologic change is present in the biopsy material.

Ringsted[3] reported the development of paresis in rats after 4-5 months on a vitamin E-free diet. This was based on direct observation of the animals and no more intimate studies were made of the musculature. The animals used in our experiments gave no such outward signs of muscular weakness, even though the maximum power of the muscles was considerably reduced.

Burr, Brown and Mosely[4] have described paralysis in rats after 22 months on a vitamin E-free diet. Their remark that the muscles are unaffected is difficult to evaluate as it is not clear whether any quantitative measure of the response of the muscle to stimulation was made. Certainly our animals have given unmistakable functional and chemical evidence of dystrophic changes in the muscle after 5 months.

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* We are indebted to Dr. K. M. Brinkhous for the preparation and interpretation of the tissue sections.

[3] Ringsted, A., *Biochem. J.*, 1935, **29**, 788.

[4] Burr, G. O., Brown, W. R., and Mosely, R. L., Proc. Soc. Exp. Biol. and Med., 1937, **36**, 780.

In view of the consistent differences in tension found between the responses to direct and motor nerve stimulation the sensitivity of the muscles to remote acetylcholine injection was tested in a group of experimental and control animals. In no instance was there any indication of a response to 400 gamma of acetylcholine bromide injected into the jugular vein of atropinized, eserinized control males while in the majority of experimental males a slight but definite response was elicited. The exact significance of this is not clear. So far as we are aware, the only procedure which will definitely sensitize skeletal muscle to acetylcholine is motor denervation. On that basis the acetylcholine test would indicate that the peripheral motor nerve is only slightly affected.

The data concerning the experimental and control females show the same changes as those of the males. Quantitatively the changes are less. It appears that there is a definite sex difference in the effect of deficient vitamin E intake upon the skeletal musculature. Whether this marked difference in the effect upon males and females is due to a gonadal or somatic effect of the deficiency is not known. It may be noted that the effect upon size is more pronounced in the males than in the females. The growth-promoting effect of vitamin E has been shown by Evans[5] to be independent of any effect upon the gonads.

Conclusions. Rats reared from birth on a vitamin E-deficient diet show dystrophic changes of the skeletal musculature within 6 months. This is evidenced by a decreased maximal contractile power, and decreased creatine and increased chloride concentration of the gastrocnemius muscle. The male is affected to a greater extent than is the female. These changes become manifest in the presence of only minor histologic change in the muscle, and without the appearance of gross symptoms. It is apparent that wheat germ oil will protect completely against these changes.

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[5] **Evans, H. M.,** ***J. Nutrition,*** **1928, 1, 23.**

9977

IV. Negative Effect of Colloidal Carriers on Enhancement of Antigenic and Sensitizing Properties of Polysaccharides.

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The rôle played by inert colloidal substances on the enhancement of antigenic properties of bacterial polysaccharides is still a debatable one. Although positive evidence of such an increase in immunological activity has been reported by Zozaya[1] and by Hoffstadt and Clark,[2] the general opinion[3, 4, 5] is that such an effect occurs with polysaccharides that are weakly antigenic themselves. In view of these discrepancies it appears to be of interest to determine whether the sensitizing and antigenic properties of the polysaccharides of *Bacillus rhinoscleromatis* can be improved by colloidal carriers. The polysaccharides of this organism were thought to be especially suitable for this study because of 2 serologically very similar preparations, one is weakly antigenic in rabbits and weakly sensitizes guinea pigs, while the antigenic properties of the other are insignificant.[6]

The polysaccharides used for this experiment include a fraction prepared by hydrolysis with 1% acetic acid and a fraction prepared by hydrolysis with 0.5% potassium hydroxide of the organism remaining after acid-hydrolysis. The details of the method of preparation and the chemical and immunological characteristics of the 2 fractions have been previously reported.[6] The colloidal substances included collodion particles, aluminium hydroxide, charcoal, and hog serum. The collodion particles were prepared according to the methods of Zozaya[1] and the aluminium hydroxide according to the method of Hektoen and Welker.[7] Adsorption was carried out by mixing varying concentrations of polysaccharide with the colloidal substance, incubating at 56°C for 2 hours and leaving in the icebox overnight. In the case of hog serum equal volumes of a 1:250

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[1] Zozaya, J., *J. Exp. Med.*, 1932, **55**, 324; *Ibid.*, 1933, **57**, 21.

[2] Hoffstadt, R. E., and Clark, W. M., *J. Inf. Dis.*, 1935, **56**, 250.

[3] Lewis, J. J., *Ibid.*, 1935, **57**, 94.

[4] Landsteiner, K., and Jacobs, J., Proc. Soc. Exp. Biol. and Med., 1933, **30**, 1055.

[5] Jacobs, J., *J. Exp. Med.*, 1934, **59**, 479.

[6] Wong, S. C., Proc. Soc. Exp. Biol. and Med., 1938, **38**, 107.

[7] Hektoen, K., and Welker, W., *J. Inf. Dis.*, 1933, **53**, 309.

concentration of polysaccharide and serum were mixed and treated in the same manner. Twenty-eight normal rabbits were divided equally into 2 groups: Group I receiving the acid-prepared polysaccharide mixed with colloidal substances and Group II receiving the alkali-prepared polysaccharide. Three rabbits were used for each type of colloidal carrier in each group and 4 rabbits received only the polysaccharides. Immunization of rabbits was carried out by giving 1 cc of the colloidal suspension on 3 successive days, resting 4 days, followed by 2 more series of injections, making a total of 9 injections representing 18 mg of polysaccharides.

For sensitization of guinea pigs only aluminium hydroxide containing 9 mg per cc of the acid-prepared polysaccharide was used. Two series of 8 guinea pigs each, weighing between 300 to 350 g, were sensitized by one subcutaneous injection followed by 2 intra-abdominal injections of the acid-polysaccharide colloidal mixture and of the acid-polysaccharide alone, each animal receiving a total of 23 mg. The injections were made at 5-day intervals and the test for supersensitivity was done 15 days after the last injection. It was found that the injection of 2 to 5 mg of the acid-polysaccharide either intravenously or intracardially into the guinea pigs of both groups elicited the same type of chronic non-fatal anaphylactic response, not a single fatal outcome having been observed.

Sera obtained from rabbits immunized with the preparations adsorbed on various colloidal carriers gave the same results as sera from control animals immunized with the polysaccharides alone. There were no demonstrable antibodies in any sera of rabbits immunized with the alkali-polysaccharides; there was no increase in the complement-fixation titer beyond 1:50,000, and there was no precipitation of a 1:1000 dilution of either polysaccharide by any serum from the animals immunized with the acid-polysaccharide.

It may be concluded that colloidal carriers such as aluminium hydroxide, charcoal, collodion particles, and hog serum, play no rôle in increasing either the antigenic or sensitizing property of the polysaccharides derived from *B. rhinoscleromatis.*

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Neostibosan and Experimental Kala-Azar in Chinese Hamsters. I. Normal Hamsters.

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The problem of chemotherapy of experimental kala-azar was investigated by Smyly,[1] who used 4 drugs, to treat infected Chinese hamsters, *Cricetulus griseus*. None of Smyly's infected animals was cured of the infection, although sodium antimonyl tartrate was given in large doses for a period sufficient to effect a cure in man. Hindle, working with Johnson,[2] obtained similar negative results with neostam, novostiburea, antimosan, and antimonyl tartrate, all of which have been successfully used in the treatment of kala-azar in man. Roehl[3] studied the action of tartar emetic, antimosan, stibosan, and neostibosan, in infected European hamsters, *Cricetulus frumentarius,* and was able to keep his treated animals free of parasites for months. Recently, Kikuth and Schmidt[4] obtained similar positive results with solustibosan. The contradictory results of Smyly and Hindle on one hand and Roehl and Kikuth and Schmidt on the other, in the opinion of the last two workers, are due to the different species of animals used. Whether Chinese hamsters, infected with *Leishmania donovani* can be cured of the infection, and whether the difference in species explains the difference in results, are the problems under investigation. Neostibosan was used because it possesses high curative value in human kala-azar and can be given subcutaneously.

Experiment 1. Determination of the *maximum non-lethal dose, maximum tolerated dose,* and *universal lethal dose* of neostibosan for hamsters. By *maximum non-lethal dose* we mean the amount given as a single injection, without killing any of the animals; *maximum tolerated dose* is the amount given as a single injection, which kills most of the animals; *universal lethal dose* is the amount given as a single injection, which kills all the animals. One hundred normal hamsters were used. Seventy-two percent of these animals weighed 30 g each; the rest varied from 25 to 36 g. The animals were divided into 5 groups, each consisting of 10 males and 10 females. Each group received a single injection of one test dose. Five test doses, 0.8 g, 1.0 g, 2.0 g, 3.0 g, and 4.0 g per kg were used. Neostibosan was freshly prepared each time in a 5% sterile aqueous solution, and given subcutaneously in abdominal wall of the animal by means of a tuberculin syringe. Any death within 48 hours after injection was considered as due to the drug. This arbitrary time limit was chosen because the excretion of pentavalent compounds of antimony is approximately 50% in the first 24 hours.[5, 6, 7] Any death occurring later than 2 days after injection is not likely to be due to the direct effect of antimony. The results of the 5 test doses are given in Table I.

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[1] Smyly, H. J., *Trans. Roy. Soc. Trop. Med. and Hyg.*, 1926, **20**, 104.

[2] Quoted from [4].

[3] Roehl, W., *Ind. Med. Gaz.*, 1929, **64**, 563.

[4] Kikuth, W., and Schmidt, H., *Chinese Med. J.*, 1937, **52**, 425.

TABLE I.
Mortality Rate of Hamsters to Test Doses of Neostibosan.

Group	Dose, g per kilo	No. of animals	No. of deaths	Mortality %
A	0.8	20	0	0
B	1.0	20	0	0
C	2.0	20	5	25
D	3.0	20	13	65
E	4.0	20	20	100

Among the 5 animals in Group C which died there were 2 males and 3 females, each weighing 30 g. Among the 13 deaths in Group D there were 6 males and 7 females (11 weighing 30 g each, and 2 weighing 29 g each). It seems that males are just as susceptible to the drug as females. From the table it is evident that among the doses tried, 1.0 g per kilo is the maximum non-lethal dose, 3.0 g per kilo, the *maximum tolerated dose,* and 4.0 g per kilo, the *universal lethal dose* of neostibosan for Chinese hamsters.

Experiment 2. Relation of body weight to the *maximum non-lethal dose.* Thirty hamsters were used. The results are shown in Table II.

TABLE II.
Relation of Body Weight of Hamsters to Maximum Non-lethal Dose of Neostibosan.

Body wt. (g)	11	12	14	15	16	17	20	24	29	30	32	34	37	40
No. of animals	1	4	2	2	2	5	3	2	1	2	2	2	1	1
No. of deaths	0	0	0	0	0	0	1	0	0	0	0	0	0	0

Except for one death among the 3 animals which weighed 20 g each, the *maximum non-lethal dose* is universal to animals weighing

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[5] Weese, H., *Chinese Med. J.*, 1937, **52**, 421.

[6] Boyd, T. C., and Roy, A. C., *Ind. J. Med. Res.*, 1929, **17**, 94.

[7] Brahmachari, U. N., Chondbury, S. C., Das, J., and Sen, P., *Ind. J. Med. Res.*, 1924, **11**, 829.

TABLE III.

Cumulative Effect of Repeated Doses of Neostibosan.

Dose (mg/kilo)	200	300	400	500	600	700	800	900	1000	1200	1400	1600	1800	2000
No. of animals	20	20	20	19	19	19	19	17	17	17	15	10	5	2
No. of deaths	0	0	1	0	0	0	2	0	0	2	5	5	3	2
Mortality %	0	0	5	0	0	0	10.5	0	0	11.7	33.3	50	60	100
Cumulative mortality	0	0	5	5	5	5	15	15	15	25	50	75	90	100

TABLE IV.

Effect of a Second Injection of Maximum Non-lethal or Toxic Dose of Neostibosan.

Dose (g per kilo)	First injection			Second injection			Interval between injections in days
	No. of animals	No. of deaths	Mortality %	No. of animals	No. of deaths	Mortality %	
1	20	0	0	20	5	25	34-50
2	20	5	25	9	4	44.5	34-51
3	20	13	65	5*	5	100	34-65

*Two animals only received 2.0 g per kilo in the first injection.

from 11 g to 40 g. The single death out of 30 hamsters may be considered as an accident. There seems to be no difference, therefore, in the susceptibility of hamsters of different body weights to the *maximum non-lethal dose* as previously determined.

Experiment 3. Cumulative effect of repeated injections and the determination of the *probable therapeutic dose.* Since repeated injections of neostibosan are required to effect a cure of kala-azar, one must know whether there is increased tolerance or cumulative effect, and find out a dose suitable for repeated injection at certain intervals with least toxic effect, to the animal.

Ten males and 10 females, each weighing 20 to 27 g were used. Injections of 1% solution of neostibosan were given subcutaneously, twice a week. The doses began at 200 mg per kilo, and were increased by 100 mg steps up to 1 g per kilo, and then by 200 mg steps up to 2 g per kilo. The results are given in Table III. The mortality rate following a single injection of 2.0 g neostibosan per kilo body weight was 25%, but after injections of increasing doses given twice weekly of doses increasing up to a final dose of 2.0 g per kilo, the mortality rate increased to 100%. This strongly speaks for a cumulative effect, and against increased tolerance. One hamster out of 20 was killed when the dose reached 700 mg per kilo. For therapeutic use in infected hamsters, it would seem, therefore, that a constant dose of 400 to 500 mg per kilo body weight, given twice a week, might be regarded as the *probable therapeutic dose* of neostibosan, but various possible influences of the infected state of hamsters with kala-azar require investigation.

Experiment 4. Effect of a second injection of *maximum non-lethal dose*, and *toxic dose.* Any dose greater than the *maximum non-lethal dose* must be regarded as a *toxic dose.* The following experiment is to observe the effects of a second injection of the same *maximum non-lethal dose* and of *toxic doses* in animals which have survived a previous injection. The interval between the injections varied from 34 to 65 days. The number of animals used for the second injection was necessarily smaller than that for the first as many died of the first dose and some were otherwise lost. The results are shown in Table IV. There was a higher mortality following the second injection, even after one or 2 months, during which the animals were expected to have recovered from the previous dose. This decreased tolerance may mean that the first dose though not fatal, damaged the organism in some way so as to lower its tolerance to the subsequent injection.

Summary and Conclusions. 1. In normal Chinese hamsters it was found that the *maximum non-lethal dose* of neostibosan is 1.0 g per kilo body weight; the *maximum tolerated dose,* 3.0 g per kilo body weight; and the *universal lethal dose,* 4.0 g per kilo body weight. 2. The sex and body weight of hamsters played no significant rôle. The *maximum non-lethal dose* was found universal to animals weighing from 11 to 40 g. 3. There was no increase of tolerance after repeated injections of gradually increasing doses of neostibosan. A cumulative effect was clearly shown. A *probable therapeutic dose* was found to be about 0.4 g per kilo body weight. 4. After an animal had been poisoned by a large dose of neostibosan, the repetition of the same or greater amount even after an interval as long as 2 months usually resulted in death.

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Neostibosan and Experimental Kala-Azar in Chinese Hamsters. II. Infected Hamsters.

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This report deals with observations on the effects of neostibosan on kala-azar in Chinese hamsters. As this work was started before Part I was completed, the *probable therapeutic dose* of neostibosan suggested in that communication was not used.

Twenty-six normal and 26 infected hamsters were equally divided into 4 groups, each consisting of 13 hamsters. Groups A and C were given repeated injections of neostibosan, while Groups B and D were kept as controls. Treatment was started 47 days after the infection with kala-azar. One pércent sterile aqueous solution of neostibosan was given twice a week by means of a tuberculin syringe, subcutaneously in 7 hamsters and intramuscularly in 6 hamsters of each group. Hamsters were weighed weekly or fortnightly and the amount of neostibosan was given according to the latest weight. The first dose was 8 mg per kilo, which was continued for 6 weeks; this amount was chosen because it is effective in human kala-azar. As soon as this dose was found to be ineffective and the *maximum non-lethal* dose was found to be 1 g per kilo, the amount was raised to 200 mg per kilo and gradually to 800 mg. Altogether 32 injections, a total of 7 g per kilo, were given to each hamster in a period of 114 days.

Difference in tolerance of normal and kala-azar hamsters. The results are shown in Table I. The mortality rate is higher in Group A than in Group C which seems to indicate that tolerance for neostibosan is less in infected than in normal hamsters, but the difference is not statistically significant. No difference in mortality rate was seen between subcutaneous and intramuscular routes of administration.

TABLE I.
Tolerance of Normal and Kala-azar Hamsters for Neostibosan.

Group of animals	No. of animals	No. of deaths	Mortality %
A. Infected hamsters receiving drug	13	7	53.8
B. ” ” ” no drug	13	3	23.0
C. Normal ” ” drug	13	4	30.7
D. ” ” ” no drug	13	2	15.4

Curative value of neostibosan in hamsters infected with kala-azar. The 32 injections of neostibosan given to Group A constituted a course of treatment. Group B served as controls. During the treatment several infected hamsters died. At autopsy the size of spleen and number of parasites were found to be decreasing with the increasing intensity of treatment. When a total dose of 2.3 g per kilo was reached, parasites were absent in almost all cases in smears made from the liver pulp obtained by puncture, while numerous parasites were found in smears of splenic pulp obtained by puncture. When a total of 7 g per kilo was reached, none of the 6 surviving hamsters showed parasites in smears of splenic pulp. A further splenic puncture was done 3 weeks after completion of the treatment in 3 of the animals and none of the smears showed parasites. Without further study one would consider these 6 hamsters apparently cured.

On completion of the treatment of kala-azar hamsters in Group A, 3 out of 13 controls in Group B had died. At autopsy all were found to have big spleens and leishmania were seen in smears. Liver puncture was done on each of the 10 survivors, and all but one were found to be heavily infected. In the negative case, the animal was later sacrificed. Its spleen and liver were found to be normal in size and no leishmania could be found in smears from these organs. It appears that this animal spontaneously recovered within about 5 months after infection, although infected hamsters usually show no such tendency.[1, 2]

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[1] Smyly, H. J., and Young, C. W., PROC. SOC. EXP. BIOL. AND MED., 1924, **21**, 354.

[2] Young, C. W., Smyly, H. J., and Brown, C., *Am. J. Hyg.*, 1926, **6**, 254.

TABLE II.
Amount of Neostibosan Received by Apparently Cured Hamsters.

Hamster No.	1029	1032	1035	1036	1041	1044
Avg wt of animal, g	26	24	20	25	24	22
Total dose, mg	192.8	174.8	149.8	185.3	181.9	155.7
" " g per kilo	7.4	7.3	7.4	7.4	7.6	7.0

Further study was made of these apparently cured hamsters (Table II), except for one (1036) which was too decomposed for use. Hamsters 1029, 1032, and 1041 were sacrificed 10 days, 3 weeks, and 3 months respectively, after completion of treatment. The spleen of each of these hamsters was ground in 3 cc normal saline solution and 0.5 cc of this emulsion intraperitoneally inoculated into 6 normal hamsters. The spleens were all small and smears made from the emulsions were all negative. Two to 4 months later the normal hamsters which had been inoculated with the emulsions from hamsters 1032 and 1041 came down with kala-azar, proving that these 2 apparently cured hamsters were not actually cured. The normal hamsters which had been inoculated with the spleen emulsion of hamster 1029 never contracted kala-azar, proving that this apparently cured hamster was actually cured.

The other 2 apparently cured hamsters, 1035 and 1044, were reinoculated 2 to 4 months after completion of treatment with 0.2 cc splenic emulsion containing numerous *Leishmania donovani.* Two months later they were killed. Hamster 1035 was found to have enlarged spleen, with parasites in spleen and liver, while hamster 1044 was found to have normal spleen and liver, with no parasites in these organs. This showed that one was not reinfectable, while the other had either been reinfected or possibly suffered a relapse. It is noteworthy that only one (1029) of the 5 apparently cured hamsters was proved to be actually cured; while a second (1044) may have been cured. No difference was seen in the curative effect of neostibosan between subcutaneous and intramuscular routes.

Further observations on the curative value of neostibosan in hamsters infected with kala-azar. A new lot of 25 hamsters which had been infected with *Leishmania donovani* for 43 days were given biweekly subcutaneous injections of 1% solution of neostibosan. Nine injections were given, beginning with a dose of 200 mg per kilo, and gradually increasing to 900 mg per kilo. Four hamsters died during the course of treatment, while 21 survived the treatment, each receiving a total of about 5.0 g per kilo. All of these were apparently cured (negative liver and spleen punctures). Three of the apparently cured hamsters were sacrificed 10 days, 18 days, and 3 months respectively after completion of treatment. All the smears made directly from the liver and spleen were negative for parasites. The spleen of each of these hamsters was used for hamster inoculation. The inoculated test hamsters were sacrificed 2 to 5 months later and the results showed that 2 of the 3 apparently cured hamsters were actually cured, but not the third whose splenic emulsion, though showing no parasites on direct study, was still capable of causing kala-azar. Twelve of the apparently cured hamsters were subjected to reinoculation 3 months after completion of treatment. About 2½ months after reinoculation one of the 7 hamsters which had received 0.1 cc of the infective material, showed evidence of kala-azar, but the 6 others were free from the infection. On the other hand, 3 out of the 5 hamsters which had received 0.2 cc of the infective material, showed evidence of kala-azar which, however, failed to develop in the others. In all, 4 or 33.3% of the 12 apparently cured hamsters were reinfected, or possibly had a relapse, while 8 or 66.7% were refractory to reinfection. The reinfectability of the apparently cured hamsters varied with the reinfective dose. Among 20 normal hamsters which had been inoculated with the same infective material as that given to the 2 apparently cured hamsters in the preceding experiment and 12 in this experiment, all except one developed kala-azar at end of 2½ months. Six of the apparently cured hamsters were kept under observation for 5 months after completion of treatment and then sacrificed. Four of the 6 were found to have enlarged spleens and livers, all harboring *Leishmania donovani,* a relapse rate of 66.7%. The 2 others had normal-sized spleens and livers with no parasites in these organs, and were apparently cured (Table III).

TABLE III.
Curative Effect of Neostibosan in 26 Apparently Cured Hamsters.

Nature of test	Result	No. of animals
Hamster inoculation test	Cured	3
	Not cured	3
Reinoculation test	Reinfectable	5
	Not reinfectable	9
Observation for relapse	Relapse	4
	No relapse	2

Discussion. There were altogether 26 apparently cured hamsters among the 38 which were systematically treated. Of these apparently cured hamsters 6 were tested by hamster inoculation and 3 were found to be actually cured, giving a cure-rate of 50% among the hamsters which survived the treatment. It is obvious that for experimental purposes the most reliable criterion of cure is inoculation of a splenic emulsion into normal hamsters. The 12 hamsters which died during the course of treatment indicate a high mortality, 31.6%. As the natural death-rate of hamsters, either non-infected or infected (Table I) is considerable, it is difficult to say how far this mortality was directly attributable to neostibosan.

The range of the effective dose of neostibosan for hamsters seems to vary from 200 to 500 mg per kilo. This dose agrees well with the *probable therapeutic dose* of 400 mg per kilo proposed in Part I. The total amount of neostibosan per kilo body weight used to effect cure in a hamster is about 100 times the human dose. In view of this, the failure of Smyly and Hindle to obtain cures in their experiments was probably due to inadequate dosage and insufficient treatment, rather than to the difference in species of hamster. Even with this enormous dose the cure rate was only 50% among the hamsters which survived the treatment and were tested by hamster inoculation, much lower than the cure-rate in man. Whether or not a 100% cure rate can be achieved in experimental kala-azar is a problem for further investigation.

That 9 of the 14 hamsters apparently cured of kala-azar by neostibosan (Table III) were refractory to a second inoculation with *Leishmania donovani* is suggestive of immunity, but one can not be sure that a focus of infection was not present. The relapse rate of 66.7% is considerable, and further suggests that the treatment given was insufficient. The occurrence of spontaneous recovery is interesting, but rather infrequent. On the whole it seems that kala-azar is more difficult to treat in hamsters than in man.

Summary. Chinese hamsters infected with kala-azar seemed to have a lower tolerance for neostibosan than normal hamsters, but the difference was not statistically significant. Five to 7 g of neostibosan per kilo body-weight constituted a course of treatment. The range of the effective dose was from 200 to 500 mg per kilo. The mortality rate during treatment was 31.6%. The apparent cure-rate in hamsters surviving the treatment was 100%, but the actual cure-rate was only 50%. An inoculation test is necessary to prove actual cure. Nine out of 14 apparently cured hamsters were refractory to a second inoculation with *Leishmania donovani*. Spontaneous recovery from and relapse of kala-azar were seen in Chinese hamsters.

9980

Correlation Between Vitamin C Content and Complement Titer of Human Blood Plasma.

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Reports concerning the relationship between serum complement and the state of nutrition with regard to Vitamin C have been few and conflicting. Zilva[1, 2] showed that depletion of Vitamin C in guinea pigs had no influence on the complement content of their serum. The same conclusion was reached by Chakraborty[3] who studied the changes of serum complement in Vitamin C deficiency in relation to sex and to the state of pregnancy of guinea pigs. On the other hand, Marsh[4] reported that the serum complement of guinea pigs decreased when Vitamin C was withdrawn from their diet. The original complement concentration was restored or even exceeded by the consumption of a diet rich in Vitamin C.

We became interested in this problem following the demonstration of reducing property of a component in complement,[5] which is also a characteristic feature of Vitamin C. Furthermore it is possible that complement content might be related to resistance against infections. Therefore it is of clinical interest to see whether we can increase the serum complement of human subjects by the administration of Vitamin C which seems to play an important rôle in combating infections.

In this communication we shall report our observations on the relationship between complement and reduced Vitamin C contents of the human blood plasma. Vitamin C was determined according to Pijoan and Klemperer's modification of Farmer and Abt's Method.[6] In the determination of complement by the biological method, we have standardized our hemolysin and sheep cell suspension, so that the complement titers obtained over a long period of time may still be comparable.[7] The observations were made on 2 groups of subjects. In the first group which consists of 2 children and one

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[1] Zilva, S. S., *Biochem. J.*, 1919, **13**, 172.

[2] Zilva, S. S., *Biochem. J.*, 1936, **30**, 1419.

[3] Chakraborty, R. K., *Indian Med. Gaz.*, 1937, **72**, 23.

[4] Marsh, F., *Nature*, 1936, **137**, 618.

[5] Chow, B. F., and Wong, S. C., Proc. Soc. Exp. Biol. and Med., 1938, **38**, 120.

[6] Pijoan, M., and Klemperer, F., *J. Clin. Invest.*, 1937, **16**, 443.

[7] Chow, B. F., to be published.

woman, simultaneous determinations of reduced Vitamin C and complement were made over extended periods in which the Vitamin C intake was raised or lowered by the addition of ascorbic acid to, or its withdrawal from, a constant basal diet. During the period of study these subjects were not suffering from febrile, diarrheal, or other diseases which may also influence the metabolism of Vitamin C.

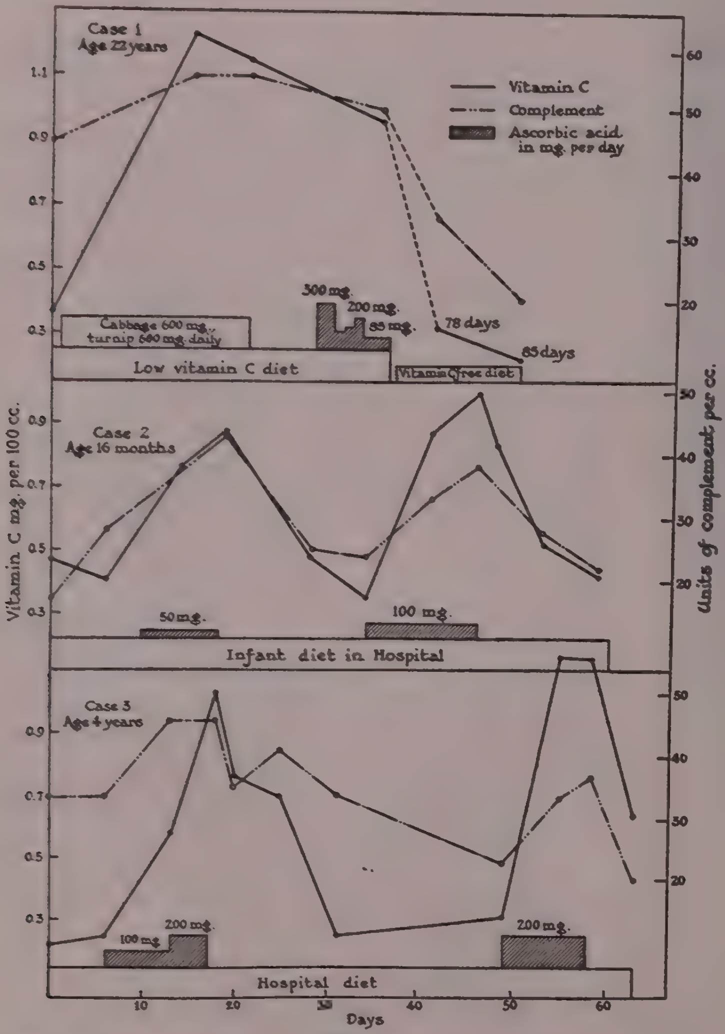

FIG. 1.
The Vitamin C content and complement titer in three normal subjects.

Results given in Fig. 1 show that in all of the 3 cases the oral administration of ascorbic acid or vegetables rich in antiscorbutic vitamin was followed by a rise in the complement as well as the Vitamin C content of the plasma, whereas their withdrawal was followed by a decrease. The range of fluctuation, however, seems to be smaller for complement than for Vitamin C. In the second group we have chosen 38 patients of different ages, suffering from different diseases and with a wide range of Vitamin C contents in their blood plasma. From Fig. 2 it may be seen that when the values of Vitamin C concentrations are divided into 3 groups of "deficiency," "presaturation" and "saturation",[8] the low Vitamin C content is in majority

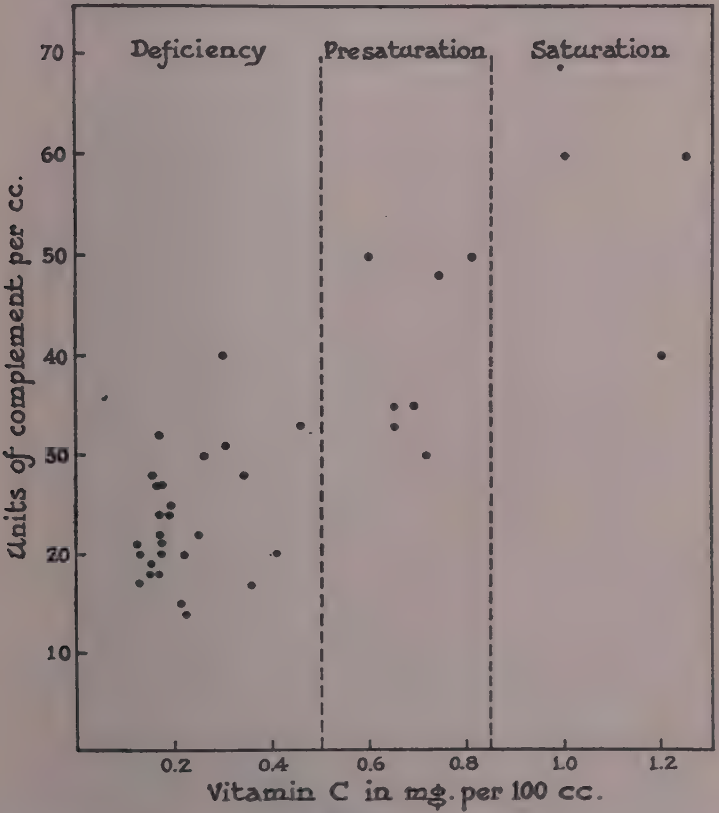

FIG. 2.

Showing the correlation of 38 simultaneous determinations of complement and Vitamin C in 30 patients.

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[8] Chu, F. T., and Sung, C., *Chinese M. J.*, 1937, **52**, 791.

of cases accompanied by a low complement titer and practically all the high Vitamin C figures are associated with high values of complement.

Conclusion. From these observations one may conclude that there is a qualitative relationship between Vitamin C intake and complement titer in the human plasma, although an adequate explanation of the phenomenon is not clear at present.

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Serological Studies on Subjects Vaccinated Against Typhus Fever.

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Since the introduction of louse vaccine by Weigl[1] for active immunization against epidemic typhus, the effectiveness of the vaccine in protecting experimental animals and human beings has been demonstrated. This has been summarized by Biraud and Deutschman.[2] In the last few years, doctors, nurses, and attendants in the Peiping Union Medical College who are likely to be exposed to typhus fever were inoculated with a louse vaccine.* This vaccine, comprising a total amount of material from 100 infected lice, was administered in 3 doses. They were given subcutaneously at weekly intervals. It was thought that it might be of interest to study the serological response of individuals after vaccination, especially in view of the scanty information of such kind in the literature. For this purpose, specimens of blood were taken by venapuncture from the vaccinated subjects at different intervals of time after the last injection. The sera so obtained were then used for agglutination against typhus Rickettsiæ and Proteus X_{19}. The agglutination test against the latter was done by the macroscopic technic of Dreyer, in which a formalinized suspension of bacteria was used as antigen. The rickettsiæ agglutination test was performed by the macroscopic technic

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[1] Weigl, R., *Med. Klin.*, 1924, **20**, 1046.

[2] Biraud, Y., and Deutschman, S., League of Nations, Epidemiological Report R. E. 183, p. 138, 1936.

* Typhus vaccine was supplied by the microbiological laboratory of Catholic University of Peking.

TABLE I.

Comparative Titer of Weil-Felix Reaction and Rickettsial Agglutination in Sera of Vaccinated Individuals.

Mo. elapsed after last injection	No. of cases	Typhus rickettsial agglutination							Weil-Felix reaction						
		No. of negatives	No. of positives						No. of negatives	No. of positives					
			1:10	1:20	1:40	1:80	1:160	1:320		1:10	1:20	1:40	1:80	1:160	1:320
½-1	45	9	18	9	5	1	1	2	9	2	9	12	6	6	1
1¼-2⅔	38	14	12	10	2	0	0	0	2	6	4	12	10	4	0
3-3½	29	10	9	10	0	0	0	0	4	3	4	10	5	3	0
4-14	9	4	5	0	0	0	0	0	1	1	2	2	3	0	0
Total, ½-14	121	37	44	29	7	1	1	2	16	12	19	36	24	13	1

of Zinsser and Castaneda.[3] The suspension was made up of 1 cc of typhus vaccine which contains material from 50 lice and 9 cc of 1:10,000 merthiolated saline. The coarse particles of the suspension were allowed to settle in the ice chest for at least 24 hours before use. The agglutination tubes were incubated in water-bath or incubator at 37°C overnight, after which readings were taken by means of a low magnifying lens (×2). The result was considered to be positive when definite clumping of the organisms was seen. Positive and negative sera were always included each time for antigen control. Serum diluted with saline was similarly incubated to serve as serum control.

The results of the comparative study of Weil-Felix reaction and typhus rickettsial agglutination are summarized in Table I.

From the table, it may be seen that while both Weil-Felix reaction and typhus rickettsial agglutination were obtained with the majority of sera of subjects shortly after inoculation, no parallelism between the titers of their respective antibodies was observed. For instance, in one serum, the rickettsial agglutinin attained a high titer of 1:320, whereas Weil-Felix reaction was positive only in 1:10. In another case, the serum agglutinated Proteus X_{19} to a titer of 1:320, while the rickettsial agglutinin was not higher than 1:20. There were also quite a number of sera which showed no agglutination for typhus Rickettsiæ (in dilution of 1:10) but gave positive Weil-Felix reaction and *vice versa*. Furthermore, although we have as yet little information of the persistence of the respective agglutinin titers, it appears that the typhus rickettsial agglutinins that have been promptly stimulated tend to wane more rapidly. On the other hand, the agglutinin titer for Proteus X_{19} seemed to persist longer.

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[3] Zinsser, H., and Castaneda, M. R., *J. Exp. Med.*, 1932, **54**, 455.

9982

Dissociation of Rickettsia and Proteus X_{19} Antibodies in Experimental Typhus Infections.

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In our studies[1] on the immune response of individuals vaccinated against typhus Rickettsiæ it was found that while the majority of the sera of these persons presented both rickettsial as well as Proteus X_{19} agglutinins, a number of them had either only one or the other. Furthermore, it has been found that the Proteus X_{19} antibodies tended to persist longer than the other. As it is difficult to study individuals repeatedly for their antibody content, and in order to find out if similar absence of correlation between the 2 antibodies is also seen in cases of actual disease, this was studied in experimental infection in animals. It has been shown[2] that *Myospalax fontanieri* differed from guinea pigs in giving, in addition to rickettsial antibodies, high Proteus X_{19} agglutinins in their serum. These animals have, therefore, been utilized in the present study, and the results herewith are communicated.

Rodents *(Myospalax fontanieri)*, which are susceptible to experimental typhus infection, have been employed. It has been found that serum from normal animals does not give a positive Weil-Felix reaction or rickettsial agglutination. The animals were infected by inoculation of brain emulsion of infected guinea pigs. At different times after the onset of fever, blood was taken by cardiac puncture, and sera examined for Proteus X_{19} and rickettsial agglutination. It was possible to bleed some animals as many as 5 to 6 times, but in the majority of cases only one bleeding was possible. Altogether 34 samples of blood were obtained from 19 animals at different times. The technic for agglutination tests has been previously described.[1] The agglutinin response of a representative animal is presented in Fig. 1 and the combined results of the experiment are summarized in Table I.

It can be seen from Fig. 1 and the table that while rickettsial agglutinin tended to appear and disappear early, the Weil-Felix reaction in the majority of the animals did not appear until after the

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[1] Liu, P. Y., Zia, S. H., and Wang, K. C., Proc. Soc. Exp. Biol. and Med., 1938, **38**, 682.

[2] Zia, S. H., and Hu, C. H., Proc. Soc. Exp. Biol. and Med., 1938, **32**, 26.

TABLE I.

Weil-Felix Reaction and Rickettsial Agglutination Produced by Serum of Rodents at Various Times During the Course of Experimental Infection.

		Weil-Felix reaction							Rickettsial agglutination				
			No. of positives at							No. of positives at			
Day of fever	Total No. examined	No. of negatives	1:10	1:20	1:40	1:80	1:160-1:320	1:640-1:1280	No. of negatives	1:10	1:20	1:40	1:80
1-2	6	6							1	1	1	2	1
2-3	4	2					2		0	1	1	2	
4-5	5	3		2					0	2	1	1	1
6-7	6	0	1	1			4		0	4	2		
8-11	3	0				1	1	1	0	1	1	1	
12-15	4	1						3	0	1	2	1	
16-19	3	0					1	2	1	1	2		
20-25	3	0					2	1	3				

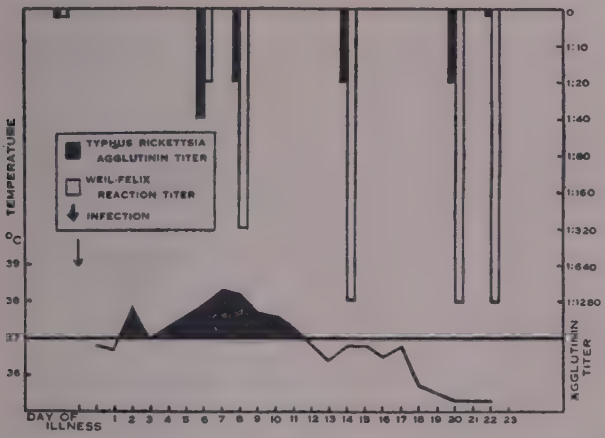

FIG. 1.

Agglutinin response in relation to the course of experimental infection in animal No. 10.

first week, and then, except for one animal, persisted long after the fever had subsided. This seems to be in general agreement with the serological results obtained in individuals following typhus vaccination.

While it is generally known that 2 distinct antibodies are produced by the Rickettsiæ, one of which is identical to an anti-Proteus X_{19}, little knowledge has been found in regard to the degree of their association during infection or after artificial immunization with rickettsial suspensions. In a previous report, we have shown that in single specimens of blood serum taken from individuals inoculated with the louse typhus vaccine, little correlation of the 2 antibodies was found. In the present communication, when repeated examinations on several individual animals were possible, a similar lack of strict parallelism was also found in experimental typhus infection. The reason for this is not clear. However, if the same principle should be applied to human cases, the rickettsial agglutinin might offer an aid to earlier diagnosis of the disease, since in experimental animals it seems to appear in most cases much earlier than the Weil-Felix reaction.

9983

Effect of Photosensitization on Immunological and Chemical Properties of Antibodies.

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While the photodynamic action of dyes was well known 30 years ago, its application to microbiology was limited to the studies on their lethal action on bacteria and protozoa, and the inactivating action on toxin, antibodies, and complement.[1] It was only in the last few years that the immunological activities of the treated substances were carefully examined. Perdrau and Todd[2] reported on the preservation of antigenicity of canine distemper virus inactivated by photodynamic action of methylene blue. Others have since studied the antigenic action of various photodynamically inactivated bacteria[3] and viruses[4] as well as toxins,[5] and in the majority of instances this has been well preserved. Furthermore, it has been suggested[3] that organisms so treated may actually make better vaccine than those treated with heat or formalin. However, the reason for this better antigenicity is not clear. It might be conceived that the less change is made in the chemical structure of the antigen, the more complete is its antigenicity. It would be difficult, however, to study chemically the change produced by photosensitization on bacteria or viruses, but it is relatively simple to study the change produced on antibodies, particularly with those in a relatively pure state. In such a case, the immunological activity will then act as an index of the photodynamic action. The result of such a study is herewith presented.

The general technic of photosensitization used in this study is essentially the same as that previously reported[3] with the exception that the immune serum was always diluted 40-100 times before exposure. Both methylene blue and eosin were employed and their effect on the immune activity of various antibodies was observed. Furthermore, the action of eosin in the presence of light on the chemical properties of a purified antipneumococcus horse precipitin[6] was also examined. It might be mentioned that the action of methylene blue and eosin was essentially similar, and that as in the case of other materials,[7] there was also an optimum for each dye in different concentrations. For instance, after 2 hours, eosin in 1:100 dilution did not exert any change in the typhoid H antibody in the presence of light, while that in 1:10,000 reduced the agglutination titer from 1:5120 to 1:640. In Table I, only the maximum effect of eosin after 120 minutes' exposure is given.

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[1] Tappeiner, H., *Erg. Physiol.*, 1909, **8**, 698.

[2] Perdrau, J. R., and Todd, C., *J. Comp. Path.*, 1933, **46**, 78.

[3] T'ung, T., Proc. Soc. Exp. Biol. and Med., 1936, **35**, 399.

[4] Galloway, J. A., *Brit. J. Exp. Path.*, 1934, **15**, 97.

[5] Lin, F. C., Proc. Soc. Exp. Biol. and Med., 1936, **34**, 656; Li, K. H., Proc. Soc. Exp. Biol. and Med., 1936, **34**, 659.

TABLE I.
Effect of Photodynamic Action on Immunological Activities.

Antibody	Immune reaction	Titer of activity	
		Exposed	Control
B. typhosus antiserum	H agglutination	1:640	1:5120
" " "	O "	1:160	1:2560
Pneumococcus type I antiserum	"	1:4	1:16
Diphtheria antitoxin	neutralization for 10 MLD	1:20	1:160
Purified antipneumococcus type I horse precipitin	mg of precipitin N necessary for the protection against 1 MLD of pneumococci	0.0625	0.016

TABLE II.
Effect of Photodynamic Action on Chemical Properties of Immunologically Pure Horse Precipitin (1 mg N/cc) of Pneumococcus Type I Polysaccharide.

	Before exposure	After exposure
Total preciptin nitrogen precipitable by its homologous polysaccharide	85%	25%
Relative viscosity in M/10 phosphate buffer at pH 6.8 at 26.5° C.	1.10	1.09
Saturation of ammonium sulfate at which precipitation of antibody protein starts	38%	20%
Solubility of antibody protein in a normal saline solution after complete precipitation at half saturation of ammonium sulfate	Complete	Complete

The deleterious effect of the photodynamic action of dyes, *e. g.*, eosin and methylene blue, on antibodies was demonstrated. The decrease in activity ranged from 4 to about 10 times. But what appears of greater interest is the relatively mild chemical changes produced on the antibody protein. It remained completely soluble in saline, and its relative viscosity was not appreciably changed from that of untreated antibody protein. The only noticeable difference lay in its solubility in various saturations of ammonium sulfate solution. The absence of any gross chemical change in spite of the marked immunological change is not surprising. It has now been repeatedly demonstrated that only slight alteration of chemical groups or of their stereo-chemical relationship may destroy all the immunological properties of protein. By analogy, it might be assumed that the protein of microörganisms subjected to the same action may also be little changed. But in this case, the preservation of antigenicity would be more complete. This may explain why organisms treated with photosensitization would make better vaccine.

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[6] Chow, B. F., and Wu, H., *Chinese J. Physiol.*, 1937, **11**, 139.

[7] T'ung, T., and Zia, S. H., PROC. SOC. EXP. BIOL. AND MED., 1937, **36**, 326.

9984

Chemical Nature of Component Involved in the Reaction Between Iodine and Complement.

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Chow and Wong have reported[1] that iodine destroys the complement activity of normal sera and that there exists a definite stoichiometric relationship between the amounts of iodine used and complement inactivated. It seemed to be of interest to find out the chemical nature of and the component involved in this process. This may either be an addition of iodine to ethylenic linkages or replacement of hydrogen atoms attached to the carbon atoms, with the result that iodine will be bound to complement. It may also be a simple oxidation of complement by iodine, which in turn, is reduced to free unbound iodide. In order to arrive at some conclusion, we have determined quantitatively first the bound iodine, if any, to the inactivated serum protein, and secondly, the free iodine in the mixture. The present communication gives the result of such a study.

1. Determination of non-dialyzable organic iodide in the inactivated serum protein. To 50 cc of fresh hog serum (having a titer of 21 units per cc) were added 10 to 15 cc of N/10 iodine solution. One-half cc of the resulting solution was found to contain less than

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[1] Chow, B. F., and Wong, Sam C., PROC. SOC. EXP. BIOL. AND MED., 1938, **38**, 120.

one unit of complement per cc. Therefore, the inactivation of complement was almost complete. The inactivated hog serum was dialyzed in a collodion tube of small porosity against distilled water at 0°C until free from iodide. Five cc of N/10 sulphuric acid were added to effect a complete solution of the protein that was precipitated out during dialysis. The total volume was then made up to 100 cc with water, *i. e.,* two times the original volume. Three 20 cc samples were pipetted into separate nickel crucibles, and their iodine content determined according to the method of McCullagh.[2] The results are given in Table I.

TABLE I.

Determination of Iodine Bound to the Inactivated Serum Protein.

Expt	Sample of hog serum cc	Units of complement per cc	Total iodine N/10 necessary for complete inactivation, cc	Total iodine equivalent to N/10 found after dialysis, cc	% iodine dialyzed
1	50	21	7	.06	99.15
2	50	30	10	.15	98.50
3	50	35	12	.10	99.17

2. Determination of iodide in the protein-free filtrate. A typical experiment was as follows: One cc of fresh hog serum (titer of 33 units per cc) was diluted ten fold with 0.85% sodium chloride solution. To each of four 2.0 cc samples of the diluted hog serum were added 5 cc of N/10 iodine solution in distilled water. To one of the mixtures, 0.6 cc of 10% sodium chloride solution was added to make the solution isotonic, in order to determine the percentage of complement activity destroyed by this amount of iodine. To the other three was added 1 cc of 2/3 N sulphuric acid and 1 cc of 10% sodium tungstate solution. The suspensions were centrifuged and 4 cc of the supernatant were used for the determination of iodide, in the usual manner, by oxidizing it to iodate with bromine. The results of several such experiments with the sera of different species of animals are given in Table II.

3. Determination of the component which is inactivated by iodine. To different samples of 0.5 cc of iodine inactivated guinea pig or hog serum was added 0.5 cc of 1:5 each of the following: To the first sample, heat inactivated, to the second, ammonia inactivated, and to the third, yeast inactivated guinea pig or hog serum; in each case, the hemolytic power of the mixture was found to be restored. To other samples of 0.5 cc of the same was added 0.5 cc of 1:10

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[2] McCullagh, B., *J. Biol. Chem.*, 1934, **107**, 35.

TABLE II.
Determination of Iodine in the Protein Free Filtrate.

Expt	cc original serum used	Units complement inactivated	Iodine added equivalent to N/100, cc	Total iodine in supernatant equivalent to N/100, cc	% recovery of iodide
		a. Hog serum.			
1	.2	4.7	.32	.288	90
2	.2	4.7	.32	.294	92
3	.2	4.7	.32	.300	94
		b. Human serum.			
1	.5	12	.32	.266	83
2	.5	12	.32	.288	90
3	.5	12	.32	.304	95
		c. Guinea pig serum.			
1	.5	30	.32	.294	92
2	.5	30	.32	.304	95
3	.5	30	.32	.310	97

either of water soluble fraction or water insoluble fraction of the given pig or hog serum, the hemolytic power of the mixture was again restored. However, when either one of water soluble or insoluble fractions was treated with iodine, the ability of the fraction to reactivate untreated fraction was destroyed.

From the results presented in the above experiments, it seems clear that in the first place, only a small amount of iodine was found in the dialyzed serum, and that iodine could be quantitatively accounted for as iodide. Therefore we are compelled to conclude that inactivation of complement is not an addition to protein, but an oxidation reaction. In the second place, serum oxidized by iodine was found to be able to reactivate sera which have been previously inactivated by yeast[3] and ammonia.[4] Furthermore, it has also been found that when complement is inactivated by these various means, the amount of iodine reduced by the inactivated complement is the same as the original serum. Hence, it may be concluded that iodine component is qualitatively different from the third or fourth component.

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[3] Osborn, W. W. B., *Complement or Alexin*, Oxford University Press, 1937, p. 16.

[4] Gordon, J., Whitehead, H. R., and Wormall, A., *Biochem. J.*, 1926, **20**, 1028, 1036, 1044.

9985

Effect of a Stimulating Dose of Pertussis Vaccine on Children Previously Immunized.

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During the course of study on the agglutinin response to pertussis vaccination in a group of Chinese children, it was noted that in every case the agglutinin titer of the blood sooner or later reached its height and then began to decline. This observation aroused our interest to try out the effect of a stimulating dose of the pertussis vaccine on the titer of the specific agglutinin, particularly in view of the successful results of restimulation in the case of active immunization against typhoid,[1] diphtheria and tetanus.[2] Five infants varying from 9 months to a little over one year of age in whom a negative history of pertussis infection in the past is reasonably sure were chosen as subjects for study. The first 2 infants were vaccinated according to the procedure recommended by Sauer;[3] namely, bilateral injections of 1 cc each into the deltoid muscles on the first occasion followed by similar injections of 1.5 cc each one and 2 weeks later respectively. To the third infant, similar doses were given with the intervals between successive injections lengthened to 3 weeks. The fourth and fifth infants differed from the third one in that they failed to come back for the third dose of vaccine. The vaccines used were prepared from cultures of the strains of *H. pertussis* isolated from our own patients not longer than 4 months previously and kept virulent by frequent transfers on the Bordet Gengou medium plus 20% fresh, defibrinated human blood. They were standardized by turbidity method to contain approximately 10 billions of organisms per cc. Blood samples were tested at frequent intervals for the presence of specific agglutinins by means of the rapid agglutination technic advocated by Kendrick.[4]

The blood of these 5 infants taken just before vaccination gave a uniformly negative result to the agglutination test. The specific agglutinin began to appear in the blood shortly after the second or

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[1] Topley, W. W. C., and Wilson, G. S., *The Principles of Bacteriology and Immunity*, 2nd Ed., page 873.

[2] Jones, F. G., and Moss, J. M., *J. Immunol.*, 1937, **33**, 173, 183.

[3] Sauer, L. W., *J. A. M. A.*, 1932, **100**, 239.

[4] Kendrick, P. L., *Am. J. Pub. Health*, 1933, **23**, 1910.

TABLE I.
Agglutinin Titer of Blood in the Children Receiving Pertussis Vaccine.

	Case 1	Case 2	Case 3	Case 4	Case 5
Before vaccination	—	—	—	—	—
1 wk after last dose of 1st course	—	1:1,280	1:40	1:320	1:80
3 ” ” ” ” ” ” ”	1:320	1:320	1:160	not done	not done
Time of 1st stimulating dose*	7	4	17	19	19
Just before 1st stimulating dose	1:160	1:320	1:40	1:80	—
1 wk after ” ” ”	1:5,120	1:2,560	1:1,280	1:2,560	1:640
2 ” ” ” ” ”	1:2,560	1:10,240	1:2,560	1:2,560	1:640
8 ” ” ” ” ”	1:1,280	1:640	1:1,280	1:640	1:160
Time of 2nd stimulating dose†	28	27	15	15	15
Just before 2nd stimulating dose	1:320	1:160	1:640	1:320	1:80
1 wk after ” ” ”	1:2,560	not done	1:2,560	1:2,560	1:1,280
8 ” ” ” ” ”	1:2,560	1:1,280	1:1,280	1:320	1:640
Strain of *H. pertussis* used for vaccination	No. 1	No. 1, 2 and 3 combined	No. 2	No. 2	No. 2

— No agglutination.
* No. of weeks after the last dose of the initial course of vaccination.
† ” ” ” ” ” first stimulating dose.

third dose of the initial course of vaccination. There was, however, a rather wide variation in the agglutination titer for the individual cases. The titers were the same regardless of whether vaccines or living suspensions prepared from the homologous or heterogeneous strains of the organisms were used as agglutinogen, provided their concentrations were the same.

After an interval of from 4 to 19 weeks after the first course of prophylactic inoculations, when the agglutinin titer of the blood had declined considerably, bilateral injections of 1.5 cc of vaccine each were given in the deltoid regions of all the 5 infants. The results shown in Table I indicate that these injections were invariably followed by a very marked increase of agglutinin within one week far above the immediate response to the initial vaccination. After another interval of from 15 to 28 weeks, the effect of such a stimulating dose was tried for the second time with more or less similar results. Although we are aware of the fact that agglutinins do not necessarily represent immunity, this observation suggests that if a stimulating dose of pertussis vaccine is given to an already vaccinated child shortly after exposure to infection, it may increase the amount of antibody during the incubation period of the disease so as to enhance the chance of protection.

9986

A Dialyzable Component of Complement.

BACON F. CHOW AND SAMUEL H. ZIA.

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When serum of a normal guinea pig is dialyzed against a normal saline solution with a cellophane tube* the hemolytic activity of the solution decreases rapidly. A prolonged dialysis will destroy almost all of the original activity. We have now found that this loss of activity is due to the loss of one of the components of complement which passes through the membrane into the saline solution, and that the original activity may be almost completely restored by the addition of the dialyzable to the non-dialyzable fraction.

A typical experiment of the dialysis is as follows: 10 cc of fresh guinea pig serum containing 80 units of complement per cc were poured into a cellophane tube both ends of which were closed with rubber stoppers and tightened with rubber bands. The sealed tube was then suspended in a graduated cylinder containing 100 cc of a cold normal saline solution. The dialysis was allowed to take place at about 5°C. After 24 hours, the bag was opened and a half cubic-centimeter of serum was pipetted out, and it was found to contain 50 units per cc. The rest of the serum was again dialyzed with another 100 cc portion of a normal saline solution. After 24 hours another half cc of the serum was taken out and its hemolytic activity was found to be 20 units per cc. The rest of the serum was again dialyzed with a fresh volume (100 cc) of the saline solution. Two days later the bag was opened and the dialyzed serum now contained less than 10 units per cc. The total volume of serum after dialysis was about 9 cc. Therefore the decrease of activity was not due to the dilution of serum. A control sample of the same guinea pig serum was stored without dialysis in another cellophane tube and kept at about 5°C. Its complement titer dropped from 80 to 60 only in 4 days.

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* Obtainable from DuPont Cellophane Co.

It was of interest to ascertain whether the loss of activity during dialysis is due to the separation of the components of complement or to some chemical reaction such as oxidation. We have found that the original complementary activity may be restored by combining the dialyzed serum and the dialyzate. This was done as follows: To 0.5 cc of the dialyzed serum diluted 30-fold with 0.35% NaCl were added varying amounts of dialyzate, and enough normal saline solution to make a total volume of 2 cc. Five-tenths cc of hemolysin (2 units/cc) and 0.5 cc of 2% sheep cells were then added. After 30 minutes at 37°C readings were made. The results of a representative experiment are presented in Table I.

TABLE I.
Restoration of Alexin Activity by Addition of Varying Amounts of Dialyzate to 0.5 cc of 1:30 of Dialyzed Serum.

Dialyzate obtained after	Volume of dialyzate in cc						
hr	1.5	1.2	1.0	0.8	0.6	0.5	0
24	4	4	4	4	2	—	—
48	4	4	4	4	4	—	—

4 = means complete hemolysis.
2 = ” partial ”
— = ” no ”

From the results given in Table I it may be noticed (1) that since a half cc of the dialyzed serum diluted 30 times with a normal saline solution was reactivated by the addition of a small amount of the dialyzable fraction. the activity of the combined fractions therefore corresponded to at least 60 units of complement per cc, and (2) that the amount of the dialyzable component necessary for the reactivation was considerably less than that present in the original serum. As little as 0.8 cc of the greatly diluted solution was sufficient to restore activity. Therefore, one may conclude that the dialysate is a component of complement.

It is well known that certain components of complement may be altered or inactivated by the treatment of yeast,[1] ammonium hydroxide,[2] or oxidation with iodine.[3, 4] It was of interest to ascertain whether the dialyzable fraction corresponds to one of these fractions. We have tried to reactivate the complementary activity of sera treated with each one of the above 3 reagents, by adding to them varying amounts of the dialyzate; the results have been consistently negative. Furthermore, if the sera inactivated yeast, ammonium hydroxide or iodine were dialyzed separately, the resultant dialysate of each of the treated sera will reactivate the inactive dialyzed untreated serum, that of the oxidized serum being the least active. These results, therefore, indicate that the dialyzable component of complement is different from the so-called "third" or "fourth" or the oxidizable components of complement.

9987

Influence of Color Filters on Photodynamic Action of Fluorescent Dyes on Gonococcus.

T. L. CH'IN. (Introduced by S. H. Zia.)

From the Department of Bacteriology and Immunology, Peiping Union Medical College, Peiping.

In the study of photodynamic action, Tappeiner[1] found that with eosin the green rays were more lethal to paramecium than the other parts of the spectrum. Later, Eidinow[2] demonstrated, however, that with the same dye the orange and yellow rays were more effective than the others. Hertel[3] in a more careful study showed that rays with different wave-lengths had different degrees of killing effect on the eosin-sensitized *B. coli* and paramecium. Most of the studies were made only with protozoa and *B. coli,* so it was thought of interest to apply similar tests to a pathogenic bacterium. In this study we have chosen gonococcus for experiment and in addition to eosin a few of the other dyes were also employed.

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[1] Osborn, W. W. B., *Complement or Alexin*, Oxford University Press, 1937, p. 16.

[2] Gordon, J., Whitehead, H. K., and Wormall, A., *Biochem. J.*, 1926, **20**, 1028, 1036, 1044.

[3] Chow, B. F., and Wong, Sam C., PROC. SOC. EXP. BIOL. AND MED., 1938, **38**, 120.

[4] Chow, B. F., and Zia, Samuel H., *ibid.*, 1938, **38**, 695.

[1] Tappeiner, H. V., *Munch. Med. Wchnschr.*, 1900, **47**, 5.

Different dilutions of eosin, methylene blue, trypaflavine, mercurochrome, protargol, argyrol, dimethyl-paraphenylenediamine hydrochloride, and tetramethyl-paraphenylenediamine hydrochloride were made with doubly distilled water, in 10-fold dilutions from $1:10^2$ to $1:10^{10}$. The method of exposure to the ordinary light is the same as previously described.[4] However, in the experiment with filtered light the cells of the hollow-ground slides were covered with, instead of the ordinary thin cover-slips, different color-filters made by the Eastman Kodak Co. The filters used were: red (F. No. 29), orange (G. No. 15), yellow (K-1 No. 6), green (B. No. 58), light blue (H. No. 45), blue (C. No. 49), and violet (D. No. 35). At intervals of 5, 10, 15, 30, 60 and 120 minutes after exposure, samples from both exposed and unexposed specimens were plated and examined for growth after 24 hours' incubation. The results of the photodynamic action of eosin, methylene blue, trypaflavine, and mercurochrome at dilutions from $1:10^5$ to $1:10^9$ after 60 minutes' exposure both to filtered and unfiltered lights are presented in Table I while those of the other dyes are omitted since it was found that their germicidal properties were not enhanced by the action of light.

In addition to the data presented in Table I, it may be stated that with unfiltered light the effect of photodynamic action of methylene blue and trypaflavine for gonococcus was first manifested at the end of 5 minutes' exposure in a dilution of $1:10^5$ while that of eosin and mercurochrome was found to appear first at the same time in the dilutions of $1:10^2$ and $1:10^4$ respectively. At the end of 120 minutes' exposure the bacteria were almost entirely killed by all the dyes at the dilution of $1:10^9$ while in the control tubes they were only slightly attacked at the dilution of $1:10^6$.

With filtered lights, the results were more complicated, but it can be stated that the photodynamic action of all the dyes used here after

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[2] Eidinow, A., *Brit. J. Radiol.*, 1930, **3**, 113.

[3] Hertel, E., *Z. allge. physiol.*, 1905, **5**, 95.

[4] T'ung, T., and Zia, Samuel H., PROC. SOC. EXP. BIOL. AND MED., 1937, **36**, 326.

TABLE I.

Photodynamic Action of Mercurochrome, Trypaflavine, Eosin, and Methylene Blue on Gonococcus at End of 60 Minutes' Exposure to Both Filtered and Unfiltered Lights.

Name of dyes	Dilution of dyes	Exposed, filtered lights							Exposed, unfiltered light	Unexposed control
		Red	Orange	Yellow	Green	Light blue	Blue	Violet		
Methylene blue	$1:10^5$	—	—	—	—	—	—	—	—	++
	$1:10^6$	—	—	—	++	++	++	—	—	++++
	$1:10^7$	++	—	++	++++	++++	++++	++++	—	++++
	$1:10^8$	++++	+++	+++	++++	++++	++++	++++	±	++++
	$1:10^9$									
Eosin	$1:10^5$	—	—	—	—	—	—	—	—	+++
	$1:10^6$	—	+	—	—	—	+	+++	—	+++
	$1:10^7$	+++	++++	++++	—	—	++	+++	—	++++
	$1:10^8$	++++	++++	++++	++	++++	++++	++++	+	++++
	$1:10^9$									
Trypaflavine	$1:10^5$									
	$1:10^6$	—	—	—	—	—	—	—	—	+
	$1:10^7$	—	—	+++	—	++	++	—	—	++
	$1:10^8$	++	—	++++	++	++++	++++	++++	—	++++
	$1:10^9$	++++	+++	++++	++++	++++	++++	++++	—	++++
Mercurochrome	$1:10^5$									
	$1:10^6$	—	—	—	—	—	—	—	—	+++
	$1:10^7$	+++	—	—	++	++	++	—	—	++++
	$1:10^8$	++++	++++	++++	++	++	++	++++	+	++++
	$1:10^9$	++++	++++	++++	+++	+++	+++	++++	++	++++

60 minutes' exposure is almost as effective as that after 120 minutes.

From Table 1 it is clear that 3 facts stand out very obviously. First, the gonococcus is particularly sensitive to the photodynamic action of the various dyes. This is not only in sharp contrast to the gram-negative bacilli but also to the closely related species such as meningococcus and *Micrococcus catarrhalis* which are more resistant than the gonococcus.[4, 5] Secondly, the effectiveness of the absorbed light in setting up the photodynamic action of the different dyes is almost as good as that of the unfiltered light. The only exception to this is found in the trypaflavine in which the unfiltered light is particularly effective, so much so that it even can bring about a complete destruction of gonococcus at the dilution of $1:10^{10}$ at the end of 60 minutes' exposure. Lastly, each of the dyes has its special absorption-band in the spectrum. Thus for methylene blue and trypaflavine, the orange rays are the most effective and in the eosin, the green and light blue rays are the effective parts. For mercurochrome the orange, yellow, and violet rays seem to act equally well.

9988

Potassium Tellurite and Copper Sulphate in Sabouraud's Medium for Isolation of Pathogenic Fungi.

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In the isolation of pathogenic fungi, one of the principal difficulties is the occurrence of bacterial contamination. This is particularly true when dealing with scales and crusts which are usually loaded with saprophytic bacteria. In trying to overcome this difficulty we have found potassium tellurite and copper sulphate to be of value as by incorporating them into Sabouraud's medium they are found to be capable of suppressing the growth of bacteria but not that of fungi. In reviewing the literature, we have been able to find few reports in which chemicals were incorporated in Sabouraud's medium for the inhibition of contaminants. Sauthof[1] found

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[5] T'ung, T., PROC. SOC. EXP. BIOL. AND MED., 1935, **33**, 328.

[1] Sauthof, Z. G., *Dermat. Wchnschr.*, 1935, **101**, 1245.

TABLE I.
Isolation of Fungi on Plain and Medicated Sabouraud's Medium.

Materials used for cultivation	No. of specimens cultured	No. of specimens from which fungi isolated						No. of specimens from which fungus was not isolated, inoculum overgrown by bacteria		
		No bacterial contamination of inoculum			With bacterial contamination of inoculum					
		A	B	C	A	B	C	A	B	C
Scale	15	15	15	0	0	0	10	0	0	5
Crust	12	12	11	0	0	1*	6	0	0	6
Hair	23	23	23	0	0	0	18	0	0	5

A = Sabouraud's medium plus potassium tellurite.
B = ,, ,, ,, copper sulphate.
C = Plain Sabouraud's medium.
* = Unidentified gram-negative bacillus.

that with stock cultures, 1-2% of yatren in Sabouraud's medium was able to inhibit the growth of staphylococcus but not of fungi. In our study the experimental material was taken directly from lesions of fungous infections since it seemed more practical to work with actual specimens than with stock cultures. The details of this study are presented below.

Different concentrations of potassium tellurite and copper sulphate were incorporated into Sabouraud's medium on which materials such as hairs, scales, and crusts taken directly from lesions of known fungous infection were heavily inoculated. Plain Sabouraud's glucose-agar was used as control. The inoculated tubes were kept at room-temperature and observations were frequently made. The suitable concentrations of these chemicals in Sabouraud's medium for this work were found to be 0.015% and 0.05% for potassium tellurite and copper sulphate respectively. The results of the culture are presented in Table I.

Besides the data presented it may be mentioned that the fungi isolated in this study were *Trichophyton violaceum, Microsporon ferrugineum, Achorion schönleini, Epidermophyton rubrum,* and *Epidermophyton inguinale.* It was noted that *Achorion schönleini* was more resistant and could stand a higher concentration of the chemicals than the others. On the Sabouraud's medium with 0.02% of potassium tellurite, the isolation of *Achorion schönleini* from *favus scutala* was usually quite easy while with the same medium, the growth of other species from hairs or scales was frequently retarded. With copper sulphate, the *Achorion schönleini* could grow very well in a concentration of 0.06% while the others were found to grow best in the concentrations of from 0.04 to 0.05%.

From Table I, it is clear that Sabouraud's medium with the addition of potassium tellurite or copper sulphate for primary isolation of fungi is definitely superior. It not only gives more positive cultures but also saves medium and labor, as with these media it is not necessary to inoculate so many tubes from a single specimen as is usually done. With potassium-tellurite medium it was found that the initial growth of the different species of fungi all became black in color. However, on microscopic examination no morphological changes were observed. Furthermore, after transfer to plain Sabouraud's medium, all the usual colonial appearances and color-characteristics were resumed. Therefore, there is no interference with the final identification.

9989 P

Purified Protein Antigen from Brucella.

PABLO MORALES OTERO AND LUIS M. GONZÁLEZ. (Introduced by D. H. Cook.)

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Several preparations have been employed in determining allergy to the brucella group.[1-5] We have tried to obtain a standard preparation which could be measured accurately, would be a good antigen and which could be easily prepared with the least possible denaturation. This has been made possible by following a modification of Seibert's method[6] for the preparation of P.P.D. (purified protein derivative from tuberculin).

Since the brucella organisms cannot be made to grow on synthetic media, as previously demonstrated by investigators,[7] we were unable to utilize a filtrate of the cultures as with the tuberculin products.[6] We decided to start with a solution of the cellular constituents of the brucella organisms and proceed to extract and purify the protein fraction. Care was taken in selecting our methods and materials to make them as simple and economical as possible. A known strain of *Brucella abortus* (strain No. 456, National Institute of Health, Washington, D. C.) was selected for our experiment.

Large amounts of *Br. abortus* are grown on standard nutrient agar pH 7[8] in Blake bottles for 72 hours at 37°C. Approximately 5 ml of saline with 0.5% phenol are added to each bottle and left for 30 minutes in contact with the culture to loosen the cells. With sterile pipettes the phenolized saline containing the suspension of cells is removed from the bottles and the surface of the agar

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[1] McFadyean, J., and Stockman, S., Report of the Departmental Comm. appointed by Bd. of Agriculture and Fisheries. Appendix to Part I, London, His Majesty's Stationery Office, 1909.

[2] Burnet, Et., *Arch. de l'Inst. Pasteur de Tunis*, 1922, **2**, 165.

[3] Giordano, A., *J. Am. Med. Assn.*, 1929, **93**, 1957.

[4] Hershey, A. D., The chemistry of Brucella. Thesis. Mich. State College, unpublished (After Huddleson's ''Brucella infections in animals and man''), 1933.

[5] Huddleson, I. F., Johnson, H. W., and Hamann, E. E., *J. Am. Vet. Med. Assn.*, 1932, **86**, 16.

[6] Seibert, F. B., *Am. Rev. Tuberc.*, 1934, **30**.

[7] Huston, R. C., Huddleson, I. F., and Hershey, A. D., Tech. Bull. No. 136, May, 1934, Michigan State College.

[8] Wadsworth, A. B., *Standard Methods*, page 90, 1927 edition.

thoroughly washed with more phenolized saline. The organisms are recovered by centrifuging at high speed; then they are crushed with sand in an electrical grinding apparatus for about 2 hours or more. The mixture of sand and broken cells is mixed with 2 liters of 0.02 N sodium carbonate and placed in the refrigerator overnight. Next morning the alkaline suspension is filtered, first through paper and then through a Berkefeld candle. To the filtrate we add 0.5% phenol to avoid bacterial contamination during the following steps of the procedure. The filtrate is then concentrated by ultrafiltration through alundum cups impregnated with 13% gun-cotton-glacial acetic acid solution prepared according to the method described by Seibert.[6] The ultrafiltration is continued until the liquid is concentrated to about 100 ml; then it is washed by passing about 500 ml of distilled water through the alundum cup filter. The concentrated colloidal solution is then filtered through a Berkefeld candle to eliminate any insoluble material that may have sedimented during the ultrafiltration. The protein is precipitated by mixing the colloidal solution with one-fourth its volume of a 50% freshly prepared aqueous solution of trichloracetic acid, making a final concentration of 10% acid. After standing overnight in the icebox the protein precipitate is thrown down by centrifugation. Then it is washed repeatedly by centrifugation with freshly prepared 10% trichloracetic acid solution, until the washings are colorless. The protein product is then washed with large volumes of anhydrous ether by repeated triturations and centrifugations until all the acid is removed and the precipitate is completely dehydrated.

The final protein product thus obtained from *Br. abortus* is a fine light brown powder, which is not completely soluble in water, but is easily dissolved by adding a few drops of 0.1 N alkali. The solution can be neutralized with N/10 hydrochloric and it remains clear. The uniform chemical composition of the product obtained is shown in Table I.

TABLE I.

	Nitrogen % dry weight	Carbohydrate (unhydrolyzed) % dry weight
1	12.35	1.02
2	12.74	1.47
3	14.35	1.19

The chemical composition of the antigen is fairly constant, the nitrogen and polysaccharide contents of protein prepared from different batches of cell cultures varying within approximately 1 or 2%.

The antigen prepared by this method gives positive biuret and Millon's tests and a positive xanthoproteic reaction. It is not coagulated by heat. Intracutaneous test in guinea pigs which have been previously infected with different strains of brucella organisms showed that a definite skin reaction lasting more than 48 hours can be produced when 0.005 mg of the purified protein is injected. Larger doses may produce necrosis or even death of the animals, depending on the degree of sensitization. Sera of rabbits inoculated with *Brucella* give a positive precipitin test and a positive complement fixation test when purified brucella protein is used.

9990

Alteration of Chromosome Sensitivity to X-rays with NH_4OH.

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Previous experiments have led the writer to attribute the formation of chromosome abnormalities by X-rays to the action of electrons on the positively charged materials of the chromosomes.[1, 2] According to this hypothesis, it is to be expected that if the sign of the electrical charges carried by the X-ray-sensitive materials of the chromosomes could be changed, the sensitivity of chromosomes to alterations by X-rays would be very much reduced. If the pH of the medium surrounding the sensitive substance could be shifted to the alkaline side of its isoelectric point or to the alkaline side of the pK of those of its constituent groups responsible for the X-ray reaction, the ionization of these positively charged groups would be suppressed and only the negatively charged groups would be left ionized. Thus it is to be expected that penetrating bases, if they are strong enough to change the intracellular pH sufficiently, should reduce the sensitivity of the chromosomes to X-rays. Penetrating acids, on the other hand, should have no effect unless the pH was shifted to such an extent that new chromosome materials or their constituents were thus brought to the acid side of their isoelectric points and therefore rendered electropositive. Substances behaving in this manner would most likely be proteins. Furthermore, if the pH of the intracellular medium surrounding the chromosomal ma-

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[1] Marshak, A., *Proc. Nat. Acad. Sci.*, 1937, **23**, 362.

[2] Marshak, A., and Hudson, J. C., *Radiology*, 1937, **29**, 669.

terials be taken as approximately 7.0, there are only two types of proteins that would carry an appreciable positive charge at this pH, the protamines and the histones. These were also the proteins indicated as the reactive ones by calculations of the size of the "sensitive volume" of the chromosomes.[1]

To determine whether the sensitive material behaves in the manner predicted by the hypothesis, the following experiments were performed: Seedlings of *Allium cepa* (onion) were brought into atmospheres of known CO_2 concentrations where they were kept for half an hour in order to come to equilibrium, and then irradiated with X-rays in the atmospheres with which they had been equilibriated. The normal and abnormal anaphases were then counted at 3 hours after irradiation. Using 11, 20, 40, 60, and 80 volumes % CO_2, no significant effect on the sensitivity of the chromosomes was observed. The differences in the percent abnormal anaphases at the different concentrations were no greater than might be expected from errors in the determination of the dose of X-rays. Therefore, if there are any other proteins in the chromosomes capable of reacting to X-rays, they must have isoelectric points lower than that represented by the intracellular pH produced by an atmosphere of 80% CO_2.

In sharp contrast to the results obtained with CO_2, it was found that dilute solutions of ammonium hydroxide in distilled water would markedly alter the sensitivity of chromosomes to X-rays. Thirty to 40 seedlings of *A. cepa* were immersed in solutions (100 cc) of various concentrations of ammonium hydroxide for half an hour and then irradiated. Several lots were irradiated simultaneously with a control group treated only with distilled water which was used as a check on the X-ray dosage at each exposure. During the period of irradiation the seedlings were kept between filter papers, each moistened with the solution in which the seedlings had previously been immersed. After irradiation the seedlings were rinsed several times in distilled water and incubated for 3 hours. They were then fixed and the anaphases examined. Seedlings of *Vicia faba* (broad bean) were treated in a similar manner, except that they were left in the ammonium hydroxide solutions for 2 hours before irradiation to insure complete penetration since their roots are considerably thicker than those of *A. cepa* (root diameters approximately 0.8 to 1.0 mm and 0.2 to 0.3 mm respectively). Only 6 seedlings were placed in each beaker with 100 cc of solution. Throughout all of these experiments the temperature was kept at 22°-23°C. The results are shown graphically in Figs. 1 and 2, and in Tables I and III.

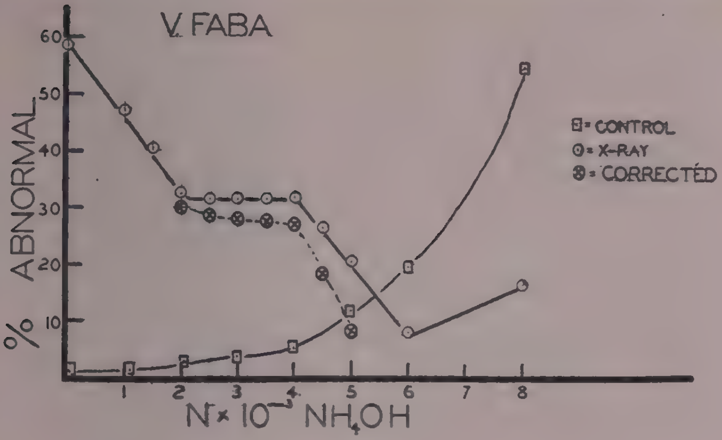

FIG. 1.
Vicia faba—100 Roentgens.

Per cent abnormal anaphases at 3 hours after irradiation with X-rays plotted against concentration of ammonium hydroxide in solutions in which the seedlings were immersed. Rectangles represent seedlings treated only with ammonium hydroxide and then incubated for 3 hours before examination. Open circles represent seedlings treated with ammonium and X-rays and then incubated. Crossed circles are obtained by subtracting the control values (rectangles) from the values after treatment with X-rays and ammonia (open circles) and represent the per cent abnormalities that may be attributed to the action of X-rays. Each point is based on counts of 150 to 1,000 anaphases except controls at concentrations 5.0 and 6.0 which are based on 68 and 97 anaphases respectively.

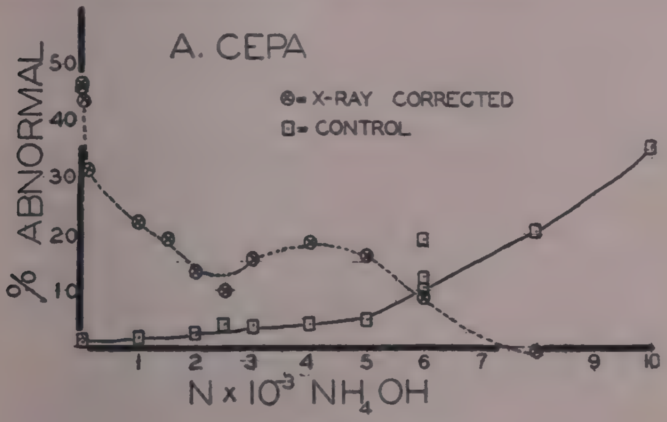

FIG. 2.
Allium cepa—80 Roentgens.

Symbols have the same significance as in Fig. 1. For simplicity the curve of open circles has been omitted. Each point is based on counts of 200 to 3,000 anaphases except control at concentration 4.0 which is based on 79.

It is evident from the figures that ammonia inhibits the formation of chromosome abnormalities by X-rays. For *V. faba*, with .002 N NH_4OH the reduction in sensitivity to X-rays amounts to a factor of approximately 2 and with .006 N to a factor greater than 60, since the corrected curve falls below 0% abnormalities at this point. Although the curve for X-rayed seedlings of *V. faba* shows a very definite plateau, the curve for seedlings treated only with ammonia gives no indication of one. With *A. cepa* the results are similar except that the curve for the X-rayed seedlings suggests that there may be a dip before the plateau is reached. More data are needed to determine whether the dip is real. The marked reduction in sensitivity by concentrations of ammonia which alone produce few or no abnormalities, and the different shapes of the X-ray and control curves indicate that the production of abnormalities by X-rays is due to their effect on a chromosomal system different from that which is altered by ammonia when it alone produces abnormalities. One effect of ammonia, and the only one at low concentrations, is to change the state of dissociation of the X-ray-sensitive materials in such a way as to render them less sensitive, presumably by changing the hydrogen ion concentration. The hypothesis that the X-ray-induced chromosome abnormalities are the result of the action of negative electrons on the positively charged groups of the dividing chromonemata is thus supported. Furthermore, since no abnormalities are produced by X-rays after treatment with .006 N NH_4OH for *V. faba* and .008 N for *A. cepa,* it would seem that at the intracellular pH corresponding to this concentration, the ionization of a large portion of the free positively charged groups is suppressed, leaving the separating chromonematic surfaces with a predominance of negative charges.* This finding would eliminate the protamines as the sensitive materials in these chromosomes, for their high content of arginine (87% of salmine, for example, is arginine) with a dissociation constant above pH 12 would require that the greater part of the X-ray-sensitive material still be positively charged and therefore capable of reacting at the high ammonia concentrations at which only a small proportion of the abnormalities may be attributed to X-rays.

Another interpretation might be given to the X-ray-ammonia curves. It is possible that ammonia might arrest mitoses at late prophase or metaphase so that the cells observed later in anaphase may have been irradiated in one of these insensitive stages rather than at the onset of prophase. One might then expect an increase of metaphases or of all division stages at the time when the cells were fixed, 3 hours after irradiation. Instead, a marked decrease in the frequency of division stages was observed. Likewise dumbbell-shaped nuclei and binucleate cells in a relatively high frequency would be expected. Only very few were found (1 to 7 per slide with 30 roots of *A. cepa* at .001 N to .006 N), and these could be shown to be the result of multiple chromosome attachments and not the result of anaphase suppression. It seems most probable, therefore, that the anaphase cells counted had their abnormalities induced at the onset of the prophase as has been demonstrated for cells not treated with ammonia, and that the ammonia reduces sensitivity at this stage.

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* At the higher concentrations of ammonia the per cent abnormal chromosomes is lower in the X-rayed seedlings of *V. faba* than in those treated with ammonia alone, which would suggest that X-rays in some way inhibit the tendency of ammonia to produce abnormalities. However, the phenomenon needs to be studied further before any very precise analysis of its nature can be made.

This conclusion is further supported by examining the percent abnormal anaphases at various intervals after immersion in ammonia. The roots examined at one-half hour after treatment with 0.0025 N NH_4OH showed the maximum number of abnormalities, which is approximately the time taken for the cell to develop from metaphase to middle or late anaphase (Table IV), indicating that the stage sensitive to the production of abnormalities by NH_4OH is the metaphase. Irradiation with X-rays produces a maximum number of abnormalities at 2½ to 3 hours after treatment, the sensitive stage being the onset of prophase, while the metaphase is quite insensitive. In producing abnormalities in metaphase, therefore, ammonia must act on quite a different chromosomal system than X-rays and also in reducing the sensitivity to X-rays at onset of prophase the ammonia must in some way be acting on the X-ray sensitive portion of the chromonema.

Ammonia has been known to produce dumbbell-shaped nuclei and

TABLE I.

A. cepa—Experiments 130, 131. X-ray—80 r. in air. Calculated Dose Received when in Chamber—75 r.

a volume % CO_2	b abnormal	c total	d % abnormal
air only	400	856	46.7
11	550	1237	44.5
20	238	565	42.2
40	324	765	42.5
60	208	449	46.4
80	206	443	46.5

TABLE II.
Summary NH_4OH—X-ray. Experiments 146, 149, 150.
V. faba—100 r. (approximate, see note below)*

a Conc. NH_4OH N $\times 10^{-3}$	Exp.	b Abnormal	c Total	d % abnormal	e % abnormal corrected for dose	f e minus control d. X-ray effect only
0	146	417	711	58.7	58.7	58.2
0	149	579	1043	55.5	58.7	58.3
0	150	264	419	63.1	58.7	58.7
1.0	146	207	439	47.2	47.2	46.5
1.5	150	461	1061	43.5	40.5	39.0
2.0	146	151	465	32.5	32.5	30.0
2.5	150	247	726	34.0	31.6	28.7
3.0	149	68	228	29.8	31.6	27.7
3.5	149	48	161	29.8	31.6	27.6
4.0	146	58	182	32.0	32.0	27.6
4.5	149	88	351	25.0	26.5	19.0
5.0	149	40	207	19.3	20.4	8.6
6.0	146	18	227	8.0	8.0	—11.6
8.0	146	38	232	16.5	16.5	—38.1
Controls—No X-ray.						
0	146	3	625	0.5		
0	149	6	1097	0.4		
0	150	—	—	—		
1.0		5	643	0.7		
2.0		11	457	2.5		
3.0		14	358	3.9		
4.0		9	203	4.4		
5.0		8	68	11.8		
6.0		29	147	19.6		
8.0		52	97	54.6		

*In experiments 149 and 150 there was a small but definite and uncontrollable fluctuation in voltage which made it impossible to get the dose exactly from dosimeter readings. The per cent abnormalities from seedlings treated only with water at each exposure has been used to obtain a correction factor to the X-ray dose value of experiment 146. The 58% abnormal for this experiment corresponds to 100 r. as determined from the survival curve by the method previously described.[2] In experiment 146 it was possible also to hold the voltage steady and the dosimeter readings gave the dose as 100 r. Voltage fluctuations did not complicate results in any of the other experiments.

binucleate cells.[3, 4] Kuwada and Nakamura[5] treated dividing plant cells with ammonia fumes and observed swelling of the chromosomes and "unraveling" of chromonemata resulting in a condition resembling a resting stage nucleus. A special effort was made while carrying out these experiments to find the swelling and "unraveling" described. At no stage after treatment with ammonia was there found to be a generalized swelling or "unraveling", although at ½ hour after treatment about half the anaphase cells had one or more attached chromosomes. When several chromosomes become

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[3] Mainx, F., *Zool. Jahrb. Abt. f. Allg. Zool. u. Physiol. der Tiere,* 1924, **41**, 553.

[4] Rosenfeld, M., *Arch. exp. Zellf.*, 1933, **14**, 1.

[5] Kuwada, Y., and Nakamura, T., *Cytologia*, 1934, **5**, 244.

TABLE III.

Summary—NH_4OH—X-ray Experiments 127, 132, 133, 148.

A. cepa—80 r.

Conc. NH_4OH N $\times 10^{-3}$	Abnormal	Total	% abnormal	d minus control d X-ray effect only
0 (127)	383	799	48.0	46.3
0 (157)	197	423	46.5	45.7
0.01	271	597	45.4	43.7
0.1	139	422	33.0	31.3
1.0	176	743	23.7	22.1
1.5	61	291	20.9	18.9
2.0	40	257	15.6	13.1
2.5	28	203	13.8	9.8
3.0	50	261	19.2	15.5
4.0	59	264	22.3	18.3
5.0	40	192	21.0	16.1
6.0	76	394	19.3	8.8
8.0	107	543	19.7	—0.8
10.0	125	435	28.7	—6.9
Controls—No X-ray.				
0 (157)	66	3953	1.7	
0 (162)	12	1530	0.8	
1.0	15	956	1.6	
2.0	6	238	2.5	
2.0	13	655	2.0	
2.5	28	696	4.0	
3.0	23	628	3.7	
4.0	3	79	3.8	
5.0	34	699	4.9	
6.0	45	248	18.0	
6.0	34	325	10.5	
6.0	34	274	12.4	
8.0	41	200	20.5	
10.0	242	681	35.6	

TABLE IV.

Anaphase Chromosome Abnormalities as a Function of Time After Treatment with 0.0025 N NH_4OH

Time after treatment in hours	0	½	1	2	3	4	5
Total anaphases	888	1107	588	547	371	473	426
% normal	68.0	51.9	87.0	92.4	95.7	95.5	96.0

so attached, the daughter anaphase groups fail to separate as sometimes happens to cells receiving heavy doses of X-rays. The observations of Kuwada and Nakamura may be interpreted, therefore, not as a direct swelling and "unraveling" produced by ammonia, but as the usual sequence of changes observed in telophase and resting stage of chromosome groups whose separation has been prevented.

Zirkle[6] has studied the relation of varying concentrations of ammonia, carbon dioxide and hydrogen sulfide on X-ray inhibition of fern spore germination and the first cell division of that spore. Curves for the effects of acids and bases alone are not given, but curves for the relative X-ray effects of the chemically treated spores as compared with irradiated, but non-treated spores are presented. Ammonia appears to inhibit the X-ray action at low concentrations, the effect reaching a maximum at about 0.003 N and then at higher concentrations, 0.008 N, markedly increases the X-ray sensitivity. The acids, on the other hand, increase sensitivity at low concentrations and decrease it at high concentrations. It is possible that the fern spore results may be related to the observations on chromosomes here presented, but there are not enough data available for the observations on the spores to show any direct correlations. In interpreting his results Zirkle assumes one or more sensitive proteins associated with the cell function studied which is normally in a medium on the alkaline side of its isoelectric point. Added acids increase sensitivity until the isoelectric point is reached and then decrease it. Although ammonia does decrease sensitivity as expected on his hypothesis, there is no adequate explanation for the observed turning point at 0.003 N, for if toxicity is to be invoked as an explanation here, it might also be used for the turning point on the acid side which is presumed to represent the isoelectric point.

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[6] Zirkle, R., *Am. J. Roent. and Radium Therapy*, 1936, **35**, 1.

The pH range included by distilled water solutions of 0.001 N to 0.01 N NH_4OH is approximately 10.27 to 10.77.[7] The absolute value of the intracellular pH is not known. However, if the internal pH is reduced by the interaction of ammonia ions with intracellular anions, the plateau of the X-ray curve might conceivably correspond with the steep portion of the dissociation curve of ammonia, where a relatively large change in ammonia normality is needed to alter appreciably the percent dissociation and hence the percent reduction in sensitivity of chromosomes to X-rays. However, if ammonia dissociation were the only factor involved one would expect a similar plateau in the ammonia control curve. There is no indication of one with *V. faba* or with *A. cepa.*

The X-ray curve for *V. faba* shows an interesting resemblance to protein titration curves and it may be possible to analyze it further in terms of the constituent amino acids of the proteins involved. As previously indicated the guanidine group of arginine with a pK of 12.5 would not be involved in the reaction while lysine with pK values 8.95 and 10.5 and cysteine with pK 8.2 and 10.3 might very well be.† Thus the chromosome response to ammonia and X-rays leads to the conclusion that the sensitive proteins are probably histones, and suggests the possibility of identifying those of its amino acid constituents which take part in the reaction. Whether these X-ray-sensitive amino acids can be more specifically and perhaps quantitatively ascertained remains for future experimentation to determine.

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[7] Michaelis, L., *Hydrogen Ion Concentration,* Baltimore, 1926, trans. by A. Perlzweig.

† Dissociation constants taken from [8].

[8] Cohn, Edwin J., *Ergebnisse der Physiologie,* 1931, **33**, 781.

Summary. Treatment with ammonia reduces the sensitivity of chromosomes to X-rays. Curves showing the decrease in sensitivity as a function of ammonia concentration are presented. The results are taken as evidence in support of the hypothesis, previously advanced, that the sensitivity of chromosomes to X-rays at the onset of prophase may be attributed to the presence of positive charges on the separating surfaces of the dividing chromonema. These separating surfaces are probably made up of proteins of the histone type.

9991

A Corpus Luteum-Stimulating Substance in the Rat Placenta.*

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In the majority of mammals the length of the pregnancy cycle exceeds that of the normal estrous or pseudopregnant cycles, and this prolongation is due in part, to a sustained function of the corpora lutea. The presence of foetal elements is in some way responsible for this continued activity of the maternal ovary.

The function of the corpora lutea of the rat during the first half of pregnancy is under the control of the hypophysis and the mechanism involved is apparently the same as that in pseudopregnancy. Luteal function is essential throughout pregnancy, however, as shown by the fact that bilateral öophorectomy invariably terminates gestation.[1-4] Removal of the pituitary gland during the first half of pregnancy is likewise followed by death and resorption of the foe-

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[*] Aided in part by a grant from the Rockefeller Foundation administered by Professor F. L. Hisaw.

[†] Rockefeller Foundation Fellow in the Natural Sciences.

[1] Harris, R. G., and Pfiffner, S. S., *Anat. Rec.*, 1929, **44**, 205.

[2] Johnson, G. E., and Challans, J. C., *Anat. Rec.*, 1930, **47**, 300.

[3] Nelson, W. O., and Haterius, H. O., *Physiol. Zool.*, 1930, **3**, 231.

[4] Hain, A. M., *Quart. J. Exp. Physiol.*, 1934, **24**, 101.

tuses, whereas pregnancy continues if hypophysectomy is performed after the tenth or eleventh day.[5, 6] In the rat hypophysectomy results in a persistence of existing corpora lutea, but such persisting corpora are non-functional in the non-pregnant animal.[7] From these considerations it is apparent that some extra-pituitary factor is responsible for the luteal function of late pregnancy.

That the uterus itself, the maternal placenta or the "metrial gland" are not concerned in this mechanism is indicated by the fact that the presence of numerous large deciduomata in the uterus fails to prolong the length of pseudopregnancy. Any influence in this regard of uterine distension or of uterine hormones is thus ruled out. Furthermore, the controlling factor is not resident in the foetuses themselves, for removal of all the foetuses by Caesarian section does not shorten the pregnancy cycle if the placentæ are left intact.[8]

Attempts to demonstrate a gonadotropic substance in the placentæ of animals other than those of certain primates and equidæ have thus far been unsuccessful, but the existence of some corpus luteum-stimulating substance of extra-pituitary source during pregnancy has been suspected by numerous investigators. These failures may be attributed to the type of tests used for the demonstration of a corpus luteum-stimulating principle.

In the present investigation the deciduoma reaction was employed as a test for corpus luteum function. Pseudopregnancy was induced by electrical stimulation of the cervix uteri during estrus and 4 days later the endometrium was mechanically traumatized. In 35 normal control animals deciduomata developed in every instance, while in 10 animals hypophysectomized on the day of endometrial trauma, the reaction did not occur. Of these latter animals, 2 were examined at 24 hours, 2 at 48 hours, 2 at 72 hours, and 4 at 96 hours; in no case was there any indication of decidual reaction, the uteri showing progressive atrophy. Being thus assured that the corpora lutea of pseudopregnancy are non-functional in the absence of the hypophysis, animals similarly prepared were used in attempts to maintain luteal function. Eleven animals hypophysectomized on the fourth day of pseudopregnancy just prior to endometrial trauma were injected twice daily with saline suspensions of fresh rat pla-

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[5] Pencharz, R. I., and Long, S. A., *Am. J. Anat.*, 1933, **53**, 117.

[6] Selye, H., Collip, J. B., and Thomson, D. L., PROC. SOC. EXP. BIOL. AND MED., 1933, **30**, 589.

[7] Greep, R. O., *Anat. Rec.*, 1938, **70**, 32; *Endocrin.*, in press.

[8] Selye, H., and McKeown, T., *Proc. Roy. Soc. London*, SB, 1935, **119**, 1.

cental tissue. In all 11 cases deciduomata were found in various stages of development when the animals were examined 24 to 96 hours after hypophysectomy. To avoid the toxicity of the fresh material various rat placental extracts were prepared and tested in the same manner. A crude alkaline aqueous extract was found to be active (2 animals). Combined isoelectric precipitates (pH 5.2 to 6.7) from aqueous alkaline extracts produced deciduomata in each of 6 animals while the concentrated supernatant fluid was inactive. Approximately one gram equivalent of fresh tissue was given daily in 2 doses. Pseudopregnant rats öophorectomized at the time of endometrial trauma did not develop deciduomata and the injection of fresh placental extracts in such animals failed to induce deciduoma formation.

Fresh placenta or the active extracts when injected into 13 normal adult females beginning on the day of estrus inhibited the ensuing cycles; deciduomata developed in all animals following uterine trauma 4 days after the beginning of injections. In these 13 animals, one gram equivalent of fresh tissue or more was given daily; smaller amounts in 17 similar animals failed to inhibit the estrous cycle and consequently no deciduomata formed. It should be noted that toxic materials are likewise capable of inhibiting the estrous cycle and permitting the development of deciduomata, rendering the non-hypophysectomized rat unreliable as a test for corpus luteum stimulation.

The length of pseudopregnancy in the rat is subject to considerable variation but in the majority of animals its duration is 12 to 14 days. If deciduomata are present in such animals their breakdown is heralded by external hemorrhage 2 to 4 days prior to the appearance of the next estrous cycle. To determine the influence of placental extracts on the prolongation of the function of the corpora lutea of pseudopregnancy, 4 animals were treated from the 8th to the 16th day of pseudopregnancy with one gram equivalent of placental extracts daily. In all 4 cases deciduomata were found at autopsy on the 17th day.

Attempts to maintain the corpora lutea of normal pregnancy beyond term have thus far been unsuccessful. The injection of large amounts of fresh placental suspension into 4 rats at frequent intervals from the 19th day of pregnancy onward did not delay parturition. Live foetuses of normal birth weight were delivered on the 21st to 22nd day. This may indicate that the termination of pregnancy is determined by a primary failure of the corpus luteum.

The active material in the rat placenta apparently is unlike any known gonadotropic substance. One gram equivalent or more injected into 21-day-old male and female rats daily for 5 days caused no increase in the weights of the ovaries, uteri, testes or seminal vesicles. As much as 5 g of fresh placenta injected intravenously into an estrous rabbit failed to induce ovulation or the formation of hemorrhagic follicles or corpora lutea. These tests establish its dissimilarity to any known gonadotropic substance of pituitary origin and differentiate it from the pregnancy hormones of the human being and the mare. Active extracts tested on 6-week-old pigeons gave negative results. Five grams equivalent in 5 daily subcutaneous injections or one-tenth gram intracutaneously over the crop gland resulted in no proliferation of the crop epithelium.

These experiments indicate that in normal pregnancy in the rat the foetal placenta secretes a substance which is responsible for the maintenance and function of the corpora lutea during the latter half of pregnancy. The functional corpora lutea induced by the act of copulation carry pregnancy to the 10th or 11th day at which time the hormone secreted by the developing chorion acts to maintain and enlarge these corpora whose continued secretion prolongs gestation to term.

In view of these and other considerations together with the available information on the chorionic hormones of the human being and the mare, it is probable that a similar mechanism is active to a greater or lesser extent in maintaining the luteal function of pregnancy in many mammals.

9992 P

Influence of Diet on Gonad Activity of English Sparrow, *Passer domesticus* (Linnaeus).

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Since the work of Rowan,[1] Bissonnette,[2] and more recently of Kirschbaum and Ringoen,[3] and others, exposure to increased light during the spring months has been considered the predominant factor in inciting the development of the gonads of birds that commonly reproduce in the spring. It is believed that the anterior hypophysis is indirectly stimulated by the increased daily light and in turn stimulates the gonads to activity.

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[1] Rowan, W., *The Riddle of Migration*, The Williams and Wilkins Co., 1931.

[2] Bissonnette, T. H., *J. Exp. Zool.*, 1931, **58**, 281.

[3] Kirschbaum, A., and Ringoen, A. R., *Anat. Rec.*, 1936, **64**, 453.

The investigators mentioned above have fairly well established the fact that increased exposure to artificial light does cause gonadal activity in such birds as juncos, starlings and English sparrows. Juvenal sparrows (birds hatched during the previous summer) thus treated in our laboratory responded after 60 days' exposure (November 15 to January 15). They were exposed to light for 10 hours daily in excess of the normal light period. The source of the light was a 25-watt Mazda lamp placed approximately one foot from the birds. The males thus treated developed the black beaks characteristic of this species during the breeding season. The testes contained cells in all stages of spermatogenesis, the sperm ducts were enlarged and otherwise functional. Female birds had, at this time, large ovaries containing numerous eggs of various sizes. The ovary had increased approximately 300% in weight and 6 times in volume over the non-treated controls. This is not the extreme increase found in fully developed females in the wild. It is, however, a marked increase, being about one-half that of the wild bird ovary at the height of development. The number of follicles, also, was greatly in excess of that of the controls. In judging the degree of ovarian development this factor is perhaps more important than weight and volume, at least in the early stages. The oviducts of light-treated birds were also greatly developed. Sections reveal the extreme epithelial and glandular development characteristic of breeding females.

In an attempt to determine what factor in the light might be responsible for these effects, birds were subjected to increased exposures of ultraviolet light during December and January. The source of such radiation was the recently developed Sperti bulb manufactured by Science Laboratories, Cincinnati, Ohio. The effects were identical to those obtained when ordinary Mazda bulbs were used. However, the time required was considerably less. The daily exposure to Sperti light was for the same length of time; comparable development of the gonads, however, was obtained in 3 weeks. It would seem, therefore, that ultraviolet radiation in nature is an important stimulating cause of gonadal activity.

Not satisfied, however, that light directly affecting the birds is the determining factor of gonadal activity for the reason that many seasonal breeding vertebrates undergo seasonal gonad activity without receiving additional amounts of light, the writer carried out the following experiments for which the light-treated birds mentioned above served as controls. Whole wheat grains were exposed to radiations from the same Sperti ultraviolet bulbs and were fed to the sparrows during December, January, and February. These birds received only the ordinary daily amount of light in the laboratory. Controls were fed on a non-irradiated wheat diet. The birds, both experimental and control, were juvenals. Three groups of experimental and control birds, 6 to a group, were used. Thus a total of 36 birds was employed. Guided by a preliminary experiment, birds were sacrificed at weekly intervals after they had received wheat irradiated for 250 hours. As soon as the wheat had been exposed to the ultraviolet light for 24 hours it was fed to the birds. Each subsequent feeding was from wheat that had received a daily increasing amount of ultraviolet light. After this exposure had reached 250 hours the gonadal response of the birds was well advanced. Birds that received wheat irradiated in excess of 300 hours showed a pronounced response. As in the case of light-treated males of both types the beaks became black, complete spermatogenesis ensued, and the sperm ducts developed to a state of functional activity. The ovaries and oviducts of the females likewise developed. Follicles in the ovaries were very numerous. Several in each ovary were more than a millimeter in diameter. Weight and volume increases were equal to or slightly greater than light-treated bird ovaries. The oviducts closely approached the condition found in oviducts of wild laying birds. That the development in both sexes was pronounced cannot be doubted when compared to birds receiving injections of Parke-Davis Antuitrin anterior lobe extract. This latter was kindly supplied by Dr. Oliver Kamm, to whom the writer is indebted. Male and female birds were injected subcutaneously with 0.2 cc of this preparation 3 times a week for 3 weeks. The response of these birds was closely comparable to the results obtained from feeding irradiated wheat. The controls of the wheat-fed birds, as well as the wild birds sacrificed during this experiment in no instance showed development beyond the resting condition of the gonads and ducts.

Conclusion. It seems likely that a chemical substance in the wheat is changed by the ultraviolet radiations. Most probably this substance directly affects the anterior hypophysis, which in turn stimulates gonad activity. If this is the case we have a new explanation of the seasonal sexual activity of the sparrow in the natural state. This interpretation, which merely shifts the emphasis from light affecting the animal to light producing a change in the diet, may well apply to the seasonal sexual development of other vertebrates as well. The results of the above experiment point to an increase of Vitamin D or some kindred substance as the specific dietary factor. Other experiments, planned to determine the exact mechanism, are in progress.

9993 P

Anomalous Behavior of Isolated Muscle Fibers Toward Certain Chemical Stimuli.

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From the Department of Zoology, University of Pennsylvania.

The rhythmic twitching induced in skeletal muscle by sodium chloride solutions is well known. It has also been known for a long time that the sodium salts of the calcium-precipitating anions induce even stronger twitching and that potassium chloride, citrate and oxalate are also effective, although potassium salts in addition cause a characteristic contracture. It was rather surprising, therefore, to find that the single isolated muscle fiber, when immersed in any of these solutions, did not twitch at all nor did it undergo contracture. The behavior of the isolated fiber was so different from that of the whole muscle that an attempt was made to discover the cause of this paradox.

Single muscle fibers were isolated from the adductor magnus muscle of the frog *(Rana pipiens)* in the usual frog Ringer's solution, buffered with di-sodium phosphate. Ordinarily, no attempt was made to preserve the nerve ending and the fiber was cut at both ends in the process of dissection. The isolated fiber then differs from the fiber in the intact muscle in that its nerve ending may be lacking, that is, it may have been located in a part of the fiber which was discarded; and also in that its ends are cut. This fiber responds quite normally to electrical and ultraviolet stimulation. It twitches in response to a condenser discharge or an induction shock and shortens on faradic stimulation or ultraviolet irradiation. However, its reaction to chemical stimulation is lacking in so far as the chloride, citrate and oxalate of sodium and potassium are concerned. Moreover, it does not respond to acetylcholine in Ringer's solution. In this respect my results corroborate those of Keil and Sichel.[1]

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[1] Keil, E. M., and Sichel, F. J. M., *Anat. Rec.*, 1937, **70** (Supplement No. 1), 112.

By more careful dissection, it was possible to isolate muscle fibers with the nerve ending intact. In the frog, the motor nerve ending is rather diffuse, so that it was not assumed to be present unless a portion of the myelinated fiber was identifiable also. Such preparations were treated with isosmotic sodium citrate or with acetylcholine in Ringer's solution (1:10,000). It was found that the presence of the nerve ending made no difference, and in no case was there any response to immersion in either of these solutions.

Fibers were also isolated intact in the semitendinosus muscle, in the manner described by Asmussen.[2] These were also immersed in isosmotic sodium citrate or acetylcholine-Ringer's solution. Again there was no reaction, although in one case, in which the fiber was immersed in acetylcholine-Ringer's solution, the nerve ending was also intact so that the fiber was exactly as in the undissected muscle. Keil and Sichel[1] concluded that the failure of the isolated fiber or small bundle of fibers to react to acetylcholine-Ringer's solution was due to the lack of the nerve ending. The results reported here do not support this conclusion because the isolated fiber does not react to this solution even when the nerve ending is seen to be present, Moreover, it is generally conceded that sodium citrate, which causes twitching in curarized muscles, acts on the muscle substance itself. Therefore, this salt should cause twitching in the isolated fiber whether the nerve ending is present or not. That it does not do so suggests that the same factor may be in operation in the failure of the isolated muscle fiber to react either to sodium citrate or to acetylcholine solutions.

Work is now in progress on the effects of various concentrations of acetylcholine and the salt solutions as well as on the effect of localized application of these solutions. The recent results of Buchthal and Lindhard[3] indicate that such localized applications of acetylcholine to the region of the motor end plate are effective in producing a twitch. However, they worked on small bundles of fibers, in which the single fibers were not completely isolated. Experiments on the size of the bundle of fibers which will react to these chemical stimulants of muscle, which will be reported in detail later, indicate that under certain conditions as few as 4 fibers, in a bundle, will react to acetylcholine or sodium citrate solutions. On the other hand, single isolated fibers have never been observed to twitch in any of the solutions used, although altogether several hundred individual fibers have been observed.

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[2] Asmussen, E., *Arch. f. d. ges. Physiol.*, 1932, **230**, 263.

[3] Buchthal, F., and Lindhard, J., *J. Physiol.*, 1937, **90**, 82P.

9994 P

Insulin Absorption from the Intestine in Normal and in Depancreatized Dogs.*

Ralph H. Major.

From the Hixon Laboratory for Medical Research, University of Kansas School of Medicine, Kansas City, Kansas.

Since the isolation of insulin, the subject of the enteral administration of insulin has excited the interest of investigators. The results reported are somewhat confusing and conflicting.

Bollman and Mann[1] found that "Large amounts of insulin may be instilled directly into the duodenum, jejunum or ileum without any appreciable effect on the sugar of the blood of normal dogs except for the slight effects when concentrated insulin was used." Bollman and Mann employed doses varying from 100 to 300 units. Murlin, Tomboulian and Pierce[2] report the absorption of insulin from the Thiry-Vella loops of the jejunum in 4 normal dogs, and in 2 of them after pancreatectomy. These observers found that the absorption was aided by the addition of 0.1% HCl, bile and hexylresorcinol.

In a large series of experiments performed during the past 3 years we have invariably obtained the results reported by Bollman and Mann when insulin in dilute acid solution was introduced into an intestinal loops of normal dogs. More recently we have obtained very definite evidence of absorption in normal and especially in diabetic dogs when the insulin was administered with glycol compounds. The most effective of these substances in our experience has been pinacol or tetramethyl-glycol. This compound is solid at room temperature and melts at 42°C. Our preparations have been prepared in the following manner:

The pinacol was melted at 42°C and added to an equal amount of insulin solution, consisting of 1000 units of insulin dissolved in 1 cc of one-tenth normal HCl. This mixture solidifies rapidly, and after standing for a brief period, it was ground into a powder, and this powder pressed into small tablets containing 50 units of insulin per tablet. In some preparations the insulin pinacol mixture was heated

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* I am under deep obligation to Eli Lilly and Company for the supply of insulin which was used in these experiments.

[1] Bollman, Jesse L., and Mann, Frank C., *Am. J. M. Sc.*, 1932, **183**, 23.

[2] Murlin, John R., Tomboulian, Ruth Latta, and Pierce, H. B., *Am. J. Physiol.*, 1937, **120**, 733.

for one-half hour at a temperature of 110°C and under 10 pounds pressure.

Some of the dogs employed in these experiments had an isolated U-loop of the jejunum, approximately 18 inches long, others a Y-type of loop, which emptied into the mid-portion of the jejunum. After experiments were performed on these normal dogs with U and Y loops, the dogs were depancreatized, and the experiments were

TABLE I.

Dog No. I. Y-loop, weight 7,900 g, pancreas removed March 5, 1938.

Normal Dog—March 1, 1938		Diabetic Dog—March 7, 1938	
Time	Blood Sugar	Time	Blood Sugar
9:30 A.M.	94	9:20 A.M.	193
	Insulin 50 units	10:00 "	Insulin 50 units
11:10 A.M.	100	1:20 P.M.	64
12:45 P.M.	98	2:40 "	49
2:50 "	91	4:00 "	55

Dog No. II. Y-loop, weight 12,050 g, pancreas removed March 24, 1938.

Normal Dog—March 23, 1938		Diabetic Dog—March 25, 1938	
Time	Blood Sugar	Time	Blood Sugar
9:00 A.M.	84	9:30 A.M.	425
9:05 "	Insulin 50 units	9:35 "	Insulin 50 units
10:30 "	93.5	11:00 "	338
12:15 P.M.	87	1:35 P.M.	322
1:30 "	85	2:05 "	384

Dog No. III. Y-loop, weight 5,950 g, pancreas removed March 29, 1938.

Normal Dog—March 25, 1938		Diabetic Dog—March 30, 1938	
Time	Blood Sugar	Time	Blood Sugar
9:00 A.M.	72	8:40 A.M.	172
9:05 "	Insulin 50 units	8:45 "	Insulin 50 units
10:50 "	75	10:30 "	134
12:00 noon	123	12:10 P.M.	159
1:30 P.M.	131	1:35 "	210

Dog No. III. U-loop, weight 10,300 g, pancreas removed March 30, 1938.

Normal Dog—March 25, 1938		Diabetic Dog—April 1, 1938	
Time	Blood Sugar	Time	Blood Sugar
9:00 A.M.	65	9:25 A.M.	425
9:30 "	Insulin 50 units	9:30 "	Insulin 50 units
10:50 "	40	11:15 "	235
12:00 noon	62	1:00 P.M.	167
1:30 P.M.	81	2:40 "	202
		4:30 "	246

Dog No. IV. Y-loop, weight 7,400 g, pancreas removed April 10, 1938.

Normal Dog—April 8, 1938		Diabetic Dog—April 12, 1938	
Time	Blood Sugar	Time	Blood Sugar
9:50 A.M.	79	9:00 A.M.	230
10:00 "	Insulin 50 units	9:30 "	Insulin 50 units
11:30 "	85.5	10:30 "	102
1:00 P.M.	78	12:00 noon	97
2:30 "	82	2:00 P.M.	169

repeated after they had become diabetic. In 37 experiments performed upon 14 dogs before and after pancreatectomy, we obtained definite evidence of the intestinal absorption of insulin in 35% of 14 experiments upon normal dogs and in 92% of 23 experiments upon diabetic dogs. In all experiments the insulin pinacol tablets were inserted into the intestinal loop and the dosage of insulin employed was 50 units. The readings in Table I are typical of the results obtained.

9995 P

Use of Phenol in Application of Prebluda-McCollum Reagent for Determining Vitamin B_1.

DANIEL MELNICK* AND HENRY FIELD, JR. (Introduced by L. H. Newburgh.)

From the Department of Internal Medicine, University of Michigan, Ann Arbor, Mich.

Recently Prebluda and McCollum[1] reported that Vitamin B_1 reacts in alkaline solution with diazotized *p*-amino acetophenone to yield a water-insoluble, purple-red compound. Subsequently, we found[2] that xylene quantitatively extracts this pigment and that the xylene layer lends itself to colorimetric evaluation. This procedure, coupled with our adsorption and subsequent elution technics using synthetic zeolite,† appears highly specific for the determination of the vitamin.

Ten cc of a thiamin hydrochloride solution at a pH of 7.0 are pipetted into a 50 cc centrifuge bottle. This is followed by the addition of 20 cc of the Prebluda-McCollum reagent. After 24 hours at room temperature 2 cc of xylene are added and the mixture shaken vigorously for 1½ minutes. After centrifugation the color in the xylene layer is compared in a micro-colorimeter with a standard similarly treated. Subsequently, in studies involving the use of phenol for extracting the vitamin from saturated salt solutions, recoveries greater than 100% were consistently obtained due to the presence of trace quantities of phenol in the final concentrates, which escaped ether extraction. Other organic hydroxy compounds, such as ethyl alcohol and pH indicators, also increase the sensitivity of the reaction.

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* Upjohn Fellow in Clinical Research, 1937-1938.

[1] Prebluda, H. J., and McCollum, E. V., *Science*, 1936, **84**, 488; *J. Biol. Chem.*, 1938, in press.

[2] Melnich, D., and Field, H., Jr., *J. Biol. Chem.*, 1938, Proc., lxxxiii.

† "Decalso," kindly furnished by the Permutit Company, New York. N. Y.

In order to investigate the behavior of phenol in this respect, a series of determinations were made in which varying amounts of phenol were added to the vitamin solutions, each 10 cc in volume and containing a total of 100 gamma of thiamin hydrochloride. No phenol was added to the standard solution. Although phenol, like some other organic compounds, reacts with the reagent to yield a colored solution, only the red pigment due to the coupled vitamin has been found to be extracted by the organic solvent. The results of these determinations are presented in Fig. 1.

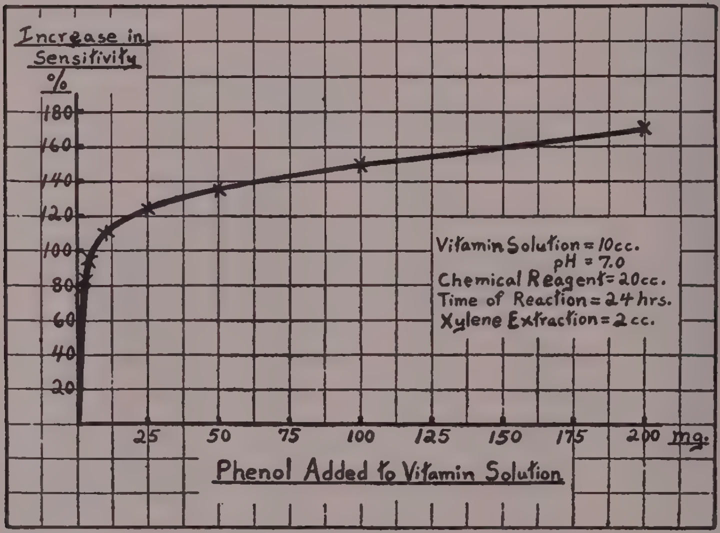

FIG. 1.

The use of phenol to increase the sensitivity of the reaction for the chemical determination of thiamin (vitamin B_1). A total of 100 gamma of thiamin hydrochloride was used in each test.

It is apparent from the curve in Fig. 1 that small quantities of phenol increase greatly the sensitivity of the reaction but with larger amounts of phenol the intensity of the color in the xylene layer becomes relatively constant. Thus small amounts of phenol, which may be present in the final solutions, have a negligible effect on the reaction when 50 mg of phenol have been added. In the presence of very large amounts of phenol (300 mg or more) the reaction fails unless larger quantities of the Prebluda-McCollum reagent are used.

With the addition of the 50 mg quantity of phenol accurate determinations were obtained on solutions ranging from 0.4 to 5 times the standard, in this case also the 10 gamma per cc solution of thiamin hydrochloride. For amounts more than 2 times the standard, greater quantities of xylene were used in order to yield solutions the color intensities of which were comparable to the standard. Without the phenol it had previously been necessary to employ a standard more closely approximating the test solution in concentration, and in some cases a previously determined reference curve had to be used in order to obtain the true values.

In these estimations of the thiamin concentrations in the presence of phenol, there was such an increase in the sensitivity of the reaction that the xylene layer was intensely pigmented. Accordingly, a micro-method was devised, using three-tenths quantities of vitamin solution, phenol and reagent, but the same volume of xylene to extract the reaction product. By this procedure it is possible to determine accurately the vitamin concentration in solutions containing as little as 10 gamma total of thiamin hydrochloride.

In addition to increasing the sensitivity of the chemical method for estimating the Vitamin B_1 concentration, the addition of phenol to the vitamin solution eliminates errors in apparent recoveries which are otherwise produced by varying the volume of the vitamin solution or the amount of the chemical reagent, allows the use of one standard solution despite very wide differences in the concentrations of the vitamin, exerts an appreciable protective action on the vitamin in solutions at a pH just alkaline to litmus, and finally eliminates the effects of trace substances which are capable of increasing the sensitivity of the reaction and which may be present in the final concentrate.

In the present paper all tests described were conducted on pure aqueous solutions of thiamin hydrochloride.[‡] This reaction can not be applied in its present form to biological materials unless quantitative procedures for the preparation of suitable concentrates are first devised. This phase of the problem is being studied.

[‡] We are indebted to the Upjohn Company, Kalamazoo, Mich., for furnishing us with a generous supply of crystalline vitamin B_1 hydrochloride.

9996

A Method of Testing Guinea Pigs for Hypersensitiveness to *Rhus toxicodendron.**

J. L. Jacobs and M. L. Welcker. (Introduced by David Rapport.)

From the Department of Pathology and Bacteriology, Tufts College Medical School, Boston, Mass.

Sensitization of guinea pigs to *Rhus toxicodendron* was reported by Rackemann and Simon,[1] and has been repeated by a number of workers.[2] For testing it has been customary to apply an acetone solution of the substance to the skin and to note differences in speed of appearance and intensity of reactions in treated animals and controls. While treated animals react more strongly to these extracts than untreated, the latter also give reactions which develop within 48 hours and make it difficult to interpret results. Recently, hypersensitiveness in guinea pigs was again studied by Ginsberg, Becker, and Becker[3] who, attempting to overcome this difficulty, applied several dilutions of acetone extract in the tests. Straus,[4] on the other hand, deeming the guinea pig unsatisfactory, used the *Rhesus* monkey which shows less irritation of normal skin; this finding has been utilized by Straus and Coca[5] in further experiments.

It seemed desirable to obtain a method of testing guinea pigs that would bring out more clearly differences between sensitized animals and controls. An experiment typical of results obtained is given below.

Six guinea pigs were sensitized, after clipping the hair with an electric clipper, by applying 5 drops of a 2% acetone solution of poison-ivy extract† on the back, spread with a glass rod. Ten days later the animals were tested on an untreated, clipped site (Table I)

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* This investigation was aided by a grant from the Charlton Research Fund of the Tufts College Medical School.

[1] Rackemann, F. M., and Simon, F. A., *Science*, 1934, **79**, 344.

[2] Simon, F. A., Simon, M. G., Rackemann, F. M., and Dienes, L., *J. Immunol.*, 1934, **27**, 113; Kobayashi, Y., *Jap. J. Dermat. and Urology*, 1935, **37**, 92; Simon, F. A., *J. Immunol.*, 1936, **30**, 275; Landsteiner, K., and Jacobs, J., *J. Exp. Med.*, 1936, **64**, 249; Kile, R. L., and Pepple, A. W., *J. Investigative Dermatol.*, 1938, **1**, 59.

[3] Ginsberg, J. E., Becker, F. T., and Becker, S. W., *Arch. Derm. and Syph.*, 1937, **36**, 1165.

[4] Straus, H. W., *J. Immunol.*, 1937, **32**, 241.

[5] Straus, H. W., and Coca, A. F., *J. Immunol.*, 1937, **33**, 215

† Courtesy of Dr. Arthur Coca, Lederle Laboratories.

by applying gently with a glass rod 2 drops of a 2% solution of poison-ivy extract in acetone. This was washed off in 5 hours with an aqueous solution of potassium permanganate (2.5%) containing 1 cc of N/1 sodium hydroxide per 100 cc. The site was then washed with water and dried with cotton, and readings were made the next day not less than one hour after application of a depilatory. A preliminary test before the sensitizing treatment was negative in all animals, and an incidental test 2 days after the sensitizing treatment showed that none of the animals had acquired hypersensitiveness at this stage, as would be expected.

TABLE I.
Sensitization to Acetone Extracts of *Rhus toxicodendron*.

Treatment Oct. 6	Guinea pig No.	Tested with 2 drops 2% acetone extr. Oct. 16; read the following day
5 drops 2% acetone extr.	1	pink, elevated
	2	faintly pink
	3	pale '' , slightly elevated
	4	faintly '' '' ''
	5	pale '' , elevated
	6	faintly '' , slightly elevated
None	7	negative
	8	''
	9	''

The table shows that guinea pigs sensitized and tested as described have definitely positive cutaneous reactions, whereas normal untreated animals tested simultaneously are negative. Controls, when this method is used, will not show a reaction at any time. If the toxic principle is not destroyed, irritation almost invariably develops within 48 hours after application of the 2% extract.

With this technic there appears to be no difficulty in carrying out sensitization experiments with *Rhus toxicodendron* in guinea pigs. The strength of the solutions and the time interval used may be varied considerably.

Preliminary experiments suggest that the principle utilized—namely, the application of a strong solution of the test substance for a short time, at the end of which the substance is destroyed or washed off, might serve to improve other tests for hypersensitiveness.

9997

Blood Sugar and Chloride Changes in Adrenalectomized Rats During Adaptation to Various Stimuli.

Hans Selye.

From the Department of Anatomy, Histology and Embryology, McGill University, Montreal, Canada.

Previous experiments have shown that if rats are continuously exposed to a uniform damaging stimulus (a drug, exposure to cold, excessive muscular exercise, etc.) they first display the symptoms of the alarm reaction,[1-5] then they become resistant to this stimulus, but finally their acquired resistance is lost again. Since this reaction and all its accompanying symptoms are almost identical in every case, and relatively independent of the specific nature of the agent to which adaptation occurs, it has been regarded as the somatic expression of adaptation as such, irrespective of the type of adaptation which is acquired. The 3 stages of this "general adaptation syndrome" have been termed: 1. The acute stage, or stage of the alarm reaction. 2. The stage of resistance. 3. The stage of exhaustion.[6-8] As a working hypothesis, it has been assumed that every organism possesses a certain limited amount of adaptability or "adaptation energy." During the alarm reaction, this would be mobilized in some way and during the stage of resistance, it would be specifically directed against the damaging agent to which the organism is exposed. The final stage of exhaustion could then be regarded as due to the depletion of the available adaptation energy.

It has been found that during the alarm reaction, the blood sugar decreases, following a transitory rise, so that marked hypoglycemia ensues. During the resistant stage, the blood sugar increases above normal, while during the stage of exhaustion, a second period of hypoglycemia ensues. The total blood chlorides decrease during

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[1] Harlow, C. M., and Selye, H., Proc. Soc. Exp. Biol. and Med., 1937, **36**, 141.

[2] Selye, Hans, *Brit. J. Exp. Path.*, 1936, **17**, 234.

[3] Selye, H., Harlow, C. M., and Collip, J. B., *Endokrinologie*, 1936, **18**, 81.

[4] Selye, Hans, *Lancet*, 1936, 1210.

[5] Schacher, Josephine, Browne, J. S. L., and Selye, H., Proc. Soc. Exp. Biol. and Med., 1937, **36**, 488.

[6] Selye, Hans, *Endocrinology*, 1937, **21**, 169.

[7] Selye, Hans, *Nature*, 1938, **141**, 926.

[8] Selye, Hans, *Nature*, 1936, **138**, 32.

the alarm reaction, rise above normal in the resistant stage and tend to decrease again during the stage of exhaustion.[9, 10]

Since the adrenal glands show marked signs of increased activity during the alarm reaction and the stage of exhaustion, but not during the stage of resistance, and since the hypoglycemia and hypochloremia characteristic of the first and third stage of the adaptation syndrome are typical of adrenal insufficiency, it was felt that a condition of relative adrenal insufficiency exists during these two stages. It seemed of interest, therefore, to study the blood chemical changes during the adaptation syndrome in adrenalectomized animals.

Female hooded rats weighing between 125 and 140 g were used. Accessory adrenals are extremely rare in our colony and adrenalectomy is almost invariably fatal within a few weeks. The animals were fed a uniform purina diet and given tap water without the addition of NaCl throughout the experiment but food was withdrawn 24 hours before the determination. In every case, the animals were given a rest of 24 hours following adrenalectomy as it was found that the blood sugar reaches a constant level at this time and stays constant until insufficiency symptoms appear. Treatment was begun in all our experiments 24 hours after the operation, so that the first day of the experiment (day 1 on the graphs) corresponds to the second day after adrenalectomy. Graph I shows the average blood sugar (continuous line) and blood chloride (dotted line) values in 3 groups of rats, each consisting of 6 animals. The initial point corresponds to the beginning of treatment in the subsequent series, that is, it represents the values 24 hours after the operation. The second point is a control corresponding to the experimental animals on the second day and the last point on the fifth day of treatment. Since the average blood sugar (Shaffer, Hartmann and Somogyi method) was 64 in the first, 66 in the second and 65 mg/100 cc in the third group, it is obvious that no significant change occurs in untreated adrenalectomized rats between the first and the sixth day after the operation. The blood chlorides (Van Slyke method), on the other hand, showed a slight linear decrease from 401 to 384 mg %. Whole blood chlorides were determined in preference to plasma chlorides as signs of intravital hemolysis have occasionally been observed during the adaptation syndrome. The possible objection that whole blood chlorides are entirely dependent on the red cell volume can be refuted, since we

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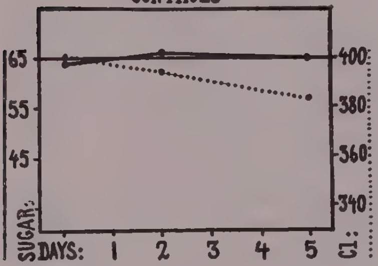
[9] Selye, Hans, Congress of Am. Physiol. Soc., Baltimore, March, 1938.

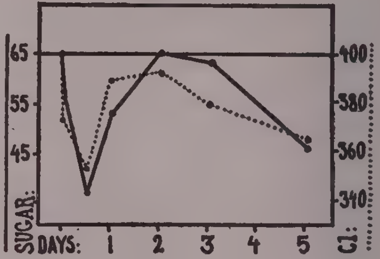
[10] Selye, Hans, *J. Gen. Physiol.*, 1938, in press.

found that although high hematocrit values are usually observed in animals in which the blood chlorides are low, there is no close correlation between these two values.

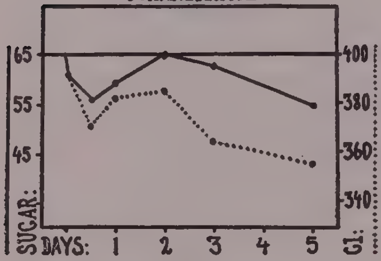
I.
CONTROLS



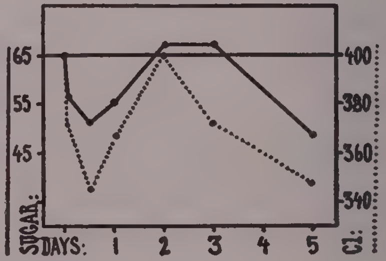
II.
EXERCISE



III.
FORMALDEHYDE



IV.
COLD



Our results are summarized in Graphs II, III, and IV. In order to avoid complicating our experiments with repeated fasting and bleeding, each rat was used for one determination only. The animals were bled in groups of 6 at 1, 12, and 24 hours and 2, 3, and 5 days respectively in each experiment. Each point on the graphs represents the average of 6 individual determinations on 6 rats. In the case of the first experiment (Graph II), the animals were adapted to muscular exercise, inasmuch as they were forced to run 3 times daily for a period of 15 minutes in revolving cages, having a diameter of 12 inches and turning at a speed of 15 revolutions per minute. In the second series, the animals were given 3 daily subcutaneous injections of 0.1 cc of a 2% formaldehyde solution, and in the third series they were exposed 3 times daily to a temperature of +1°C for one hour each time.

As can be seen from the graphs, the blood sugar and blood chloride curves run approximately parallel in all these experiments, reaching a first minimum level after 12 hours' treatment, a maximum after 48 hours, and a second minimum on the fifth day. It should also be mentioned that the appearance of the animals ran closely parallel with these blood chemical findings, inasmuch as the general condition was particularly bad during the first day, almost normal, in spite of continued treatment, on the second and third day, while on the fifth day, they appeared even more seriously damaged than on the first day.

Although no accurate blood volume determinations were made, it became obvious from the amount of blood obtained by cutting the carotid that the blood volume likewise parallels the blood sugar and chloride values and so does the volume of the spleen.

Both the experiments described in the literature and the present findings, we feel, are in agreement with the conception[11, 12] that the adrenals play an important rôle in the process of adaptation. Although adaptation is possible in the complete absence of adrenal tissue, it cannot be maintained for a prolonged period, that is to say, the stage of resistance of the general adaptation syndrome is particularly short. The simplest explanation of these phenomena would be that the organism uses so much of its "adaptation energy" for adaptation to life without the adrenals that little is left for resistance against damaging agents. As a result of this, the appearance of the signs of the alarm reaction and the stage of exhaustion (decrease in blood sugar, blood chlorides, blood volume, thermoregulating ability, muscular efficiency, general resistance, etc.), all of which are also known as characteristic signs of adrenal deprivation, may readily be accelerated in adrenalectomized rats by exposure to damaging agents.

Recently an increase in blood potassium has frequently been regarded as the primary change in adrenal insufficiency. Kendall and Ingle[13] showed, however, that while not pretreated adrenalectomized animals are extremely sensitive to potassium, they can acquire an adaptation to it if they are pretreated with gradually increasing doses. In this case, they will withstand large amounts of potassium although their blood potassium rises to a very high level. It is evident from these findings that the mere presence of large amounts of potassium in the blood cannot in itself be the cause of the adrenal insufficiency symptoms, but potassium intoxication, like any other condition of stress, is not tolerated well by adrenalectomized animals unless they have previously been adapted to it.

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[11] Selye, Hans, *Science*, 1937, **85**, 247.

[12] Selye, Hans, *Arch. Internat. de Pharm.-dynamie et de Thér.*, 1937, **55**, 431.

[13] Kendall, E. C., and Ingle, D. J., *Science*, 1937, **86**, 18.

Summary and Conclusions. Experiments in the rat show that continuous exposure to non-specific damaging agents elicits a characteristic 3-stage general adaptation syndrome which is of considerably shorter duration in the adrenalectomized animal than in the normal. During the first stage, the blood chlorides and blood sugar are decreased; during the second stage, both these values return to normal in spite of continued treatment and the general condition of the animals is considerably improved. During the final third stage of exhaustion, hypoglycemia and hypochloremia reappear and general resistance decreases again. These experiments are in agreement with the conception that the primary cause of the adrenal insufficiency syndrome is a disturbance in the mechanism of adaptation and more particularly a decrease in the ability to maintain adaptation once it is acquired. The decrease in blood sugar, blood chlorides, blood volume, thermoregulating ability, muscular efficiency, etc., as well as the increase in blood potassium, are probably all simply the consequences of this primary disability.

9998 P

In vitro and *In vivo* Effect of Sulfanilamide on *Brucella abortus* and *Brucella suis*.

BEN D. CHINN. (Introduced by S. A. Koser.)

From the Department of Bacteriology, Medical College of the State of South Carolina, Charleston.

In vitro Experiments. Various concentrations of para-aminobenzene sulfonamide* were added to dextrose broth, veal-infusion broth, and 10% serum-veal-infusion broth. The effect of the chemical on the growth-rates of *Br. abortus* and *Br. suis* was relatively the same regardless of the medium used.

The strains used had been under artificial cultivation for over 2 years but were still pathogenic for guinea pigs. The cultures grew quite well on veal-infusion agar and in the various liquid media mentioned above after incubation for 48 hours at 37°C under ordinary atmospheric conditions.

One-tenth cubic centimeter of a 1:100 dilution of a 48-hour broth culture was seeded into flasks containing 100 cc of broth; sterile

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* The para-aminobenzene sulfonamide (prontylin) was kindly supplied by the Winthrop Chemical Co., New York.

1% solution of *p*-aminobenzene sulfonamide was added to obtain concentrations of 1:1000, 1:10,000, 1:100,000, and 1:1,000,000. Control cultures did not contain any sulfanilamide.

Plate counts were made before the sulfanilamide was added, 10 minutes after its addition and after 24, 48, and 72 hours' incubation at 37°C. The initial counts were between 1000 and 3000 organisms per cc. Within 10 minutes after the sulfanilamide was added a marked reduction in the number of viable cells was found in the 1:1,000, 1:10,000, and 1:100,000 dilutions. All the organisms were destroyed during this short period in the 1:1000 dilution, for no colonies appeared on plates subsequently made from both the *Br. abortus* and *Br. suis* cultures in this dilution. Most of the organisms were destroyed in the 1:10,000 dilution after 10 minutes' exposure and those remaining were either killed or inhibited by the drug after further incubation. After 72 hours the counts were less than 10 organisms per cc.

In the 1:100,000 dilution the bactericidal effect was less marked but there was a definite bacteriostatic effect throughout the incubationary period. The count after 72 hours was 35,000 per cc for *Br. abortus*. With the *Br. suis* strain there were 500 cc after 24 hours and none after 72 hours. The 1:1,000,000 dilution was slightly bactericidal after 10 minutes and definitely bacteriostatic thereafter. There were 1,200,000 organisms per cc in the *Br. abortus* culture and 2,,500,000 in the *Br. suis* culture after 72 hours. The control cultures contained 3,000,000 and 45,000,000 organisms per cc for *Br. abortus* and *Br. suis* respectively after 72 hours.

These experiments demonstrate the bactericidal and bacteriostatic effect of *p*-aminobenzene sulfonamide on *Br. abortus* and *Br. suis in vitro*. Similar results have more recently been obtained with *Br. melitensis*.

In vivo Experiments. *Br. abortus* and *Br. suis* infections in guinea pigs generally produce a chronic type of disease if small doses are inoculated. In 4 to 6 weeks the most characteristic pathological change is an enlarged, nodular spleen and a nodular liver but lesions may also be found in the testicles, kidneys, and other organs. The invading bacteria can usually be isolated from these tissues.

In order to determine the protective effect of *p*-aminobenzene sulfonamide in experimental infections, 6 guinea pigs were inoculated intraäbdominally with a culture of *Br. abortus* and 6 with *Br. suis*. One-half cc of an agar-slant culture suspended in 5 cc of saline was injected into each animal.

Three animals in each group were given a single oral dose of

100 mg of sulfanilamide daily. Treatment was started 2 hours after inoculation. The other 3 guinea pigs, used as controls, were not treated. After 30 days the 12 animals were killed, the gross lesions noted and cultures made from livers and spleens.

The spleens of the untreated guinea pigs were, in most cases, large and nodular. The spleens of the 6 treated animals appeared normal on gross examination and their average weight was 0.87 g. The average weight of 5 normal guinea pigs' spleens was 0.98 g. The average weight of the spleens from the 6 untreated guinea pigs was 1.54 g. During the 30-day period the loss of weight of the untreated animals was somewhat greater than that of the treated animals. Cultures from the spleen and liver of the treated guinea pigs were negative while cultures of Brucella were obtained from all of the untreated animals.

From these results it is apparent that the oral treatment of Brucella infections in guinea pigs with *p*-aminobenzene sulfonamide is effective in preventing a generalized infection when treatment is begun immediately after infection. It appears from these experiments that most or all of the organisms are destroyed before any extensive invasion can take place or that the sulfanilamide inhibits the multiplication of the organisms in the tissues.

The therapeutic effect of *p*-aminobenzene sulfonamide in Brucella-infected guinea pigs is under investigation.

9999 P

Effect of Sodium Citrate and Sodium Bicarbonate on Ethyl Alcohol Acidosis.

BYRON B. CLARK AND ROBERT W. MORRISSEY. (Introduced by H. E. Himwich.)

From the Department of Physiology and Pharmacology, Albany Medical College, Albany, New York.

It has been shown by Himwich, *et al.*,[1] that an acidosis follows the ingestion of large quantities of ethyl alcohol. This paper reports the action of sodium citrate and sodium bicarbonate on the changes in alkaline reserve and blood lactic acid resulting from large doses of ethyl alcohol in dogs.

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[1] Himwich, H. E., Nahum, L. H., Rakieten, N., Fazekas, J. F., DuBois, D., and Gildes, E. F., *J. Am. Med. Assn.*, 1933, **100**, 651.

The dogs were given orally 5 g per kg of ethyl alcohol diluted to 20% with water. In 12 control experiments, determinations of alcohol,[2] carbon dioxide combining capacity,[3] and lactic acid[4] were carried out on arterial blood before and at 2-hour intervals for 10 to 16 hours, and in 4 experiments at the end of 24 hours after the alcohol administration. Similar blood analysis on the dogs to receive the alkalizing salts were carried out for 6-10 hours after the alcohol administration, at which time sodium citrate or sodium bicarbonate was given. The observations were then continued for the subsequent 4 to 8 hours, and at the end of 24 hours. In 5 experiments, sodium citrate was given orally in a dosage of 0.15 g per kg, and in 2 experiments sodium bicarbonate 0.45 g per kg.

A significant degree of acidosis was observed. It was noted that 6-10 hours following the alcohol ingestion, the carbon dioxide capacity was decreased on the average by 4-5 volumes %, but returned to the original level after 24 hours. Blood lactic acid increased during the same time interval by an average of 8 mg % or approximately 50%; even after 24 hours it was still 3.5 mg % above the average starting value. The blood alcohol had decreased during this period from about 0.6% to approximately 0.1%. Alkalizing salts strikingly reduced the elevated blood lactic acid. Within 2-4 hours after sodium citrate was given, the lactic acid decreased to or below its original value and so continued during the remainder of the experiment. The effect of sodium bicarbonate was more prompt and marked, the lactic acid decreasing within 4 hours to 10 mg % below the average control level. After 24 hours it was still 8 mg % below the average control level for animals receiving alcohol but no sodium bicarbonate. The blood alcohol was approximately 0.45% at the time the sodium bicarbonate was given. The reduced carbon dioxide capacity was elevated within 1 to 2 hours by the sodium citrate and sodium bicarbonate to approximately the original level or slightly above. (The effect of sodium citrate is summarized in Fig. 1) Sodium citrate and sodium bicarbonate had no influence upon the rate of disappearance of ethyl alcohol from the blood.

The effect of alkalizing salts in restoring the normal blood lactic acid is particularly significant. It seems probable that this may be accomplished by facilitating the conversion of lactic acid to glycogen by the liver. Since an alkalosis will itself cause a rise in blood

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[2] Harger, R. N., *J. Lab. and Clin. Med.*, 1935, **20**, 746.

[3] Van Slyke, D. D., and Neill, J. M., *J. Biol. Chem.*, 1924, **61**, 523.

[4] Friedmann, T. E., Cotonio, M., Shaffer, P. A., *J. Biol. Chem.*, 1927, **73**, 335.

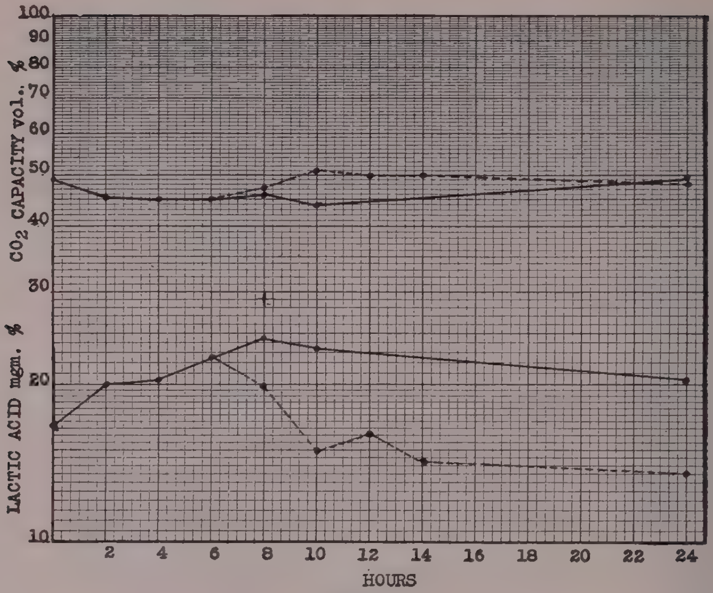

FIG. 1.

Effect of Sodium Citrate on Alcohol Acidosis.

Changes in blood lactic acid and plasma CO_2 capacity are plotted on semi-logarithmic paper.

——— Effect of alcohol 5 g per kg. Average of 12 experiments for 6-10 hr and 4 experiments at 24 hr.

------- Effect of sodium citrate 0.15 g per kg on the alcohol acidosis. Average of 5 experiments.

lactic acid,[5] only minimal amounts of alkali were given. The alkaline reserve was promptly increased. After prolonged alcoholic intoxication with some depletion of body base, this would be even more important. Thus the administration of sodium citrate or sodium bicarbonate in the treatment of the acidosis following large amounts of alcohol is a rational procedure.

Summary. Alcohol given in doses of 5 g per kg to dogs resulted in a decrease in carbon dioxide-combining capacity, and a prolonged elevation of blood lactic acid. Sodium citrate or sodium bicarbonate in the dosages used restored the alkaline reserve to approximately the normal level, and lowered the blood lactic acid below its initial value even in the presence of a moderately high blood alcohol concentration. Sodium citrate and sodium bicarbonate had no effect upon the rate of disappearance of alcohol from the blood.

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[5] Macleod, J. J. R., and Knapp, H. J., *Am. J. Physiol.*, 1918, **47**, 189; Anrep, G. V., and Cannan, R. K., *J. Physiol.*, 1923-24, **58**, 244.

10000

Maintenance of Spermatogenesis in Hypophysectomized Mice with Androgenic Substances.*

W. O. NELSON AND C. E. MERCKEL.

From the Department of Anatomy, Wayne University College of Medicine.

It was shown by Walsh, Cuyler and McCullagh[1] and confirmed by Nelson and Gallagher[2] that spermatogenesis could be maintained in hypophysectomized rats by the administration of male hormone extracts. These studies were extended by Nelson and Merckel[3] and Nelson[4] through the use of various crystalline androgens. Since these studies had been confined to rats it was of interest to extend them to other species. This report deals with results obtained upon mice. Studies upon guinea pigs are in progress.

Adult male mice were hypophysectomized by the parapharyngeal approach. The effect of hypophysectomy upon the reproductive organs in the mouse have been described by Leblond and Nelson.[5] Injections of androgenic hormone† were begun on the second day after hypophysectomy and continued for 20 days. The animals received the routine colony ration with the addition of sugar in their drinking water In the averages only those animals are included that survived through the experimental period and in which hypophysectomy was shown to have been complete by serial section of their sellæ turcicæ. At the time of autopsy the testes, seminal vesicles, prostate, and adrenals were weighed. These and the epididymis, Cowper's glands, vas deferens and thyroid were fixed for histological study. Smears of the epididymal head and tail as well as of the vas deferens were studied for the presence and motility of sperm.

Table I includes the data of studies thus far completed and is given in the form of averages. In the table a group of 5 animals

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* Aided by a grant from the Committee on Scientific Research of the American Medical Association.

[1] Walsh, E. L., Cuyler, W. K., and McCullagh, D. R., *Am. J. Physiol.*, 1934, **107**, 508.

[2] Nelson, W. O., and Gallagher, T. F., *Science*, 1936, **84**, 230.

[3] Nelson, W. O., and Merckel, C. G., PROC. SOC. EXP. BIOL. AND MED., 1937, **36**, 825.

[4] Nelson, W. O., *Cold Spring Harbor Symposia on Quantitative Biology*, 1937, **5**, 123.

[5] Leblond, C. P., and Nelson, W. O., *Compt. rend. Soc. biol.*, 1937, **124**, 9.

† Generously supplied by Dr. Erwin Schwenk of the Schering Corporation.

TABLE I.

Male Mice Hypophysectomized for 22-23 Days and Treated for 20-21 Days with Androgenic Substances. Weights Are Given as Averages.

No. of Animals	Body weights (first) g	Body weights (last) g	Treatment Daily dose in mg	Organ weights (g) Testes	Organ weights (g) Seminal Vesicles (full)	Organ weights (g) Seminal Vesicles (empty)	Prostate	Sperm Motility
15	28.5	29.6	Normal controls	.211	.243	.118	.048	++++
9	27.7	22.3	Hyp. controls	.031	—	.017	.012	No Sperm
3	27.6	22.4	Androstanedione, 0.8	.198	.149	.087	.041	++++
4	27.0	22.3	,, 0.6	.186	.128	.074	.039	++++
4	27.0	21.8	,, 0.4	.140	.114	.057	.029	++++
4	27.5	22.4	Androstanediol, 0.8	.175	.263	.135	.052	++++
3	28.2	23.1	,, 0.4	.123	.205	.121	.044	++++
3	28.3	23.4	Testosterone propionate, 0.8	.168	.579	.158	.085	++++
5	28.6	23.6	,, ,, 0.4	.120	.409	.118	.067	++++
4	26.3	21.5	Testosterone, 1.0	.116	.218	.107	.049	++++
3	28.1	23.4	,, 0.5	.109	.189	.095	.043	++++
5	27.4	26.9	Androgenic hormone*	.164	.198	.091	.053	++++

*Three animals received androstanedione, one received testosterone propionate, and one received testosterone.

are included in which small fragments of anterior lobe tissue were found. It will be noted that although the body weights of these animals were about the same at the close of the experiment as they had been at the beginning, thus indicating the presence of sufficient anterior lobe tissue to maintain body weight, this residual tissue did not enhance the effect of the treatment with androgenic substance. This is noteworthy since Gomez, *et al.*,[6] have indicated that small fragments of pituitary will entirely alter the effect of estrogenic substance in the mouse. We have been unable to detect any evidence of such enhancement in these studies.

It is apparent from the table that the results on mice confirm those previously obtained with rats. Androstanedione proved to be the most effective of the androgens in maintaining spermatogenesis as well as testis weight, although it is much less active than testosterone or testosterone propionate in androgenic potency. Androstanediol was slightly less effective than androstanedione. The results have not been quite as striking, even with androstanedione, as in the rat, but the use of somewhat larger amounts of androgen may result in responses entirely comparable to those observed in the latter animal.

The microscopic findings were similar to those previously re-

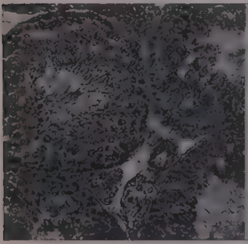

FIG. 1

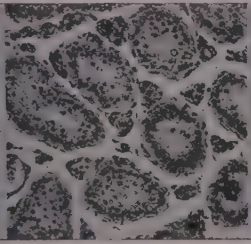

FIG. 2

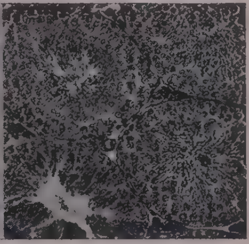

FIG. 3

FIG. 1.

Testis of normal adult mouse. Note particularly the large interstitial cells. × 116.

FIG. 2.

Testis of hypophysectomized untreated mouse. Note the extensive damage to the seminiferous tubules and the shrunken interstitial cells. × 116.

FIG. 3.

Testis of hypophysectomized mouse which had received 0.8 mg of androstanedione daily. The tubular diameter is slightly decreased, but spermatogenesis is normal. The condition of the interstitial cells is not improved by the treatment. × 116.

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[6] Gomez, E. T., Turner, C. W., Gardner, W. U., and Hill, R. T., PROC. SOC. EXP. BIOL. AND MED., 1937, **36**, 287.

ported for the rat. The testes of all animals injected with androgens showed spermatogenesis in progress, although the degree of tubular maintenance varied with the particular androgen. The latter was most marked in cases where androstanedione or androstanediol had been used. Even here, as is true of the rat, occasional tubules that show degenerative changes are encountered. The striking degeneration of the interstitial cells which follows hypophysectomy in the mouse is not prevented by treatment with androgens. Nor are the damaged adrenals and thyroid altered. As would be expected the male accessory organs present a normal or hypertrophied histological picture in animals which had received treatment. Figures 1 to 3 represent typical areas in the testes of normal, hypophysectomized-control, and hypophysectomized-treated animals.

Summary. The injection of crystalline androgenic hormones will prevent the marked degenerative changes in the seminiferous tubules of hypophysectomized mice for at least 23 days. The most effective substance used has been androstanedione. The degenerative changes in the interstitial cells, adrenals and thyroid were not altered.

10001 P

Toxicity of $MnCl_2.4H_2O$ when Fed to Rats.

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From the Biochemical Laboratory, School of Hygiene and Public Health, The Johns Hopkins University.

Findlay[1] claims that addition of manganese chloride to the "ordinary" diet of rats produces cirrhosis of the liver and death in from 6 to 10 weeks. He fed 0.3 to 0.4 g manganese chloride per rat per day but failed anywhere to indicate the composition of the diet used. McCarrison[2] fed rats 0.889 mg of MnO per day. He compared their weights with those of a similar group receiving no manganese other than that contributed by the salt mixture and the other constituents of the diet. He states, "There was no difference in the percentage weight curves of the 2 groups up to the 32nd day. Thereafter the curves diverged gradually and increasingly; that of the group receiving manganese being at the lower level." The experiment lasted 135 days. To another group of rats he fed $MnCl_2$ at a level of .0327 mg per rat per day. A control group received no $MnCl_2$. He states, " . . . from the outset the rate of growth of the group receiving 0.327 mg $MnCl_2$ daily per rat surpassed that of the control group." The experiment lasted 53 days. Unfortunately, the diets used in these 2 sets of experiments were not identical either in the source of the manganese, amounts of manganese, or sources of fat or vitamins.

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[1] Findlay, G. M., *Brit. J. Exp. Path.*, 1924, **5**, 92.

[2] McCarrison, R., *Ind. J. Med. Res.*, 1926-27, **14**, 641.

Orent and McCollum[3] demonstrated the need of the rat for manganese for normal reproduction. On manganese-free diets females fail to suckle their young, and males show testicular degeneration after 100 days or less. The addition of .005 to .05% manganese in the form of $MnCl_2.4H_2O$ promotes normal functioning of males and females.

As a result of these observations further experiments were conducted where manganese was added to the basal ration at the following levels of $MnCl_2.4H_2O$: 0.18%, 0.36%, 0.9%, 1.8%, 3.6%. Thus there was added to the diets .0499, .0998, .2495, .4990, and .9980 g of manganese respectively. The basal ration had the following composition:

	%
Yeast	10.0
Casein	18.0
Salts (No. 51)	6.1
Dextrin	57.9
Butterfat	8.0
Viosterol	15 drops per kilo of ration

Young animals weighing 40 to 50 g were taken from our stock diet and placed on the experimental rations.

On all the diets except where $MnCl_2.4H_2O$ was fed at 3.6% the animals grew well and produced healthy young. On the latter diet there was some depression of growth but here, as at the lower levels of manganese feeding, reproduction was excellent. The experiments on the 4 lower levels of manganese were terminated after the animals had been on the diet 240 days. Only the group receiving 3.6% $MnCl_2.4H_2O$ was kept for longer observation. At this level marked rancidity of the fat developed. When the ration was kept on ice no such changes occurred. Hence this practice was followed. As mentioned above, there were no indications of toxicity on this diet other than slight depression of growth. After 730 days on the diet 2 males still show normal testes and motile sperm as determined by examining epididymal smears obtained by aspiration. Each of these males has subsequently been bred to 4-month-old virgin females from our stock colony. One sired 40 young when mated with 8 females, the other 25 young when mated with 5 females. Of 50 males on similar diets but without added manganese, other than that found as a contaminant of certain inorganic constituents and natural foods as shown by Orent and McCollum,[3] testicular atrophy and tubular degeneration was marked in 45 males after they had been on the rations from 117 to 833 days.

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[3] Orent, E. R., and McCollum, E. V., *J. Biol. Chem.*, 1931, **92**, 651.

On the type of diet employed, food consumption averages from 10 to 15 g per adult rat per day. Animals on the above rations were thus consuming about .018, .036, .09, .18, and .36 g of $MnCl_2.H_2O$ per day, or .00449, .00998, .02495, .0499, and .0998 g of manganese. The rations used contained .63 g calcium and .72 g phosphorus per 100 g. Evidently the high level of phosphorus effectively prevented symptoms of toxicity by reducing the amount of absorbable manganese.

It is thus apparent that the conditions in the intestines which influence the absorbability of manganese constitute the controlling factor in determining the level at which it is toxic. From our observations on a small number of rats it also seems clear that when a high intake of this element is provided sexual function is retained in males decidedly longer than is the case in our rats on similar diets without added manganese. This observation is being checked on a larger group of male rats.

10002

Excretion of Homogentisic Acid After Oral Administration of Phenylalanine to Alcaptonuric Subjects.

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From the Departments of Biological Chemistry and Internal Medicine, University of Michigan, Ann Arbor.

That the homogentisic acid excreted in alcaptonuria originates from the aromatic amino acids of the protein molecule, tyrosine and phenylalanine, has been accepted.[1] Further confirmation has been obtained[2] by the isolation and characterization of homogentisic acid from the urine of healthy white rats fed large amounts of phenylalanine. In most of the studies concerned with the fate of the naturally occurring aromatic amino acids in alcaptonuria, tyrosine has been administered, while experiments with phenylalanine are very limited in number.[1] Since we had available for study 2 alcaptonuric patients in the University Hospital, we have undertaken further quantitative studies of the effect of phenylalanine on the excretion of homogentisic acid in alcaptonuria.

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* On leave of absence from the Medical School of Emory University.

[1] Compare Neubauer, O., *Handbuch der normalen und pathologischen Physiologie*, 1928, **5**, 851, for an excellent review of the literature.

Two patients of middle age, brothers, served as subjects. The older was admitted to the hospital because of a fractured leg and the alcaptonuria was discovered in the course of routine examination of the urine. In neither case did the patient seek medical advice because of the alcaptonuria. Homogentisic acid was determined by

TABLE I.

Effect of Oral Administration of *l*(−)-Phenylalanine on Urinary Excretion of Homogentisic Acid in Alcaptonuria.

Values are expressed as grams excreted per day. Figures in parentheses represent the range of values observed. Patient O.T. served as the subject in series A and B and patient R.T. in series C. The diet of series A and C was the standard hospital diet; that of B, an exclusive milk diet, except as noted.

Day	Homogentisic acid, g	Nitrogen, g	H:N × 100	Notes
		Series A.		
1-8	3.9	10.50	37	Control period
	(2.3-4.8)	(6.90-13.59)	(31-41)	
9	9.1	14.44	63	5 g phenylalanine*
10	13.0	10.99	118	10 ” ” *
11	4.6	12.12	38	Control day
12	4.8	12.70	38	” ”
		Series B.		
1-6†	7.1	14.50	49	Control period
	(6.3-7.6)	(14.26-14.66)	(44-52)	
7	12.1	14.40	84	10 g phenylalanine‡
8	18.0	14.10	128	15 ” ” ‡
9	9.0	11.24	80	Control day
10	7.2	14.40	50	” ”
11-15	5.1	12.40	40	” period§
	(4.4-5.8)	(11.41-13.40)	(36-42)	(hospital diet)
		Series C.		
1-7	3.90	8.90	44	Control period
	(2.8-4.9)	(7.02-10.97)	(43-45)	
8	10.3	9.10	113	10 g phenylalanine‡
9	7.70	8.19	94	10 ” ” ‡
10-13	3.10	7.60	40	Control period
	(2.3-4.1)	(5.23-9.60)	(39-43)	

*The phenylalanine was given in 2 portions, one at 7 and one at 12.
†The analyses were carried out on the last 3 days of the control period.
‡The phenylalanine was administered in a single dose at breakfast.
§Regular hospital diet.

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[2] Papageorge, E. T., and Lewis, H. B., *J. Biol. Chem.*, 1938, **123**, 211.

the method of Briggs.[3] The patients received the regular hospital diet without restriction and in one series, a diet of 3 liters of milk daily was fed (Series B). The $l(-)$-phenylalanine was administered as indicated in Table I.

It will be observed that with both patients, the ratio of homogentisic acid to nitrogen (H:N), when the usual hospital diet was fed, ranged from 31 to 42 (Subject O.T.) and 39 to 45 (Subject R.T.), values similar to previously reported values.[1, 4] When, however, the protein of the diet was derived solely from milk (Series B), the H:N ratio was higher, 44-52. This may be assumed to be due to percentages of the aromatic amino acids in the proteins of milk[5] higher than those in the proteins present in the usual mixed diet.

In Series A, 2 doses of 2.5 g each of phenylalanine were fed at 7:30 A. M. and at 12 noon respectively. Two doses of 7.5 g each were similarly ingested on the second experimental day. The conversion to homogentisic acid, as evidenced by its excretion, was practically complete on the day when the smaller doses were fed, but the extra homogentisic acid excreted on the second day, when 15 g of phenylalanine were fed, corresponded to approximately 60% of the phenylalanine. When the same subject was ingesting a milk diet, the administration of 25 g of the amino acid over a period of 2 days (Series B) resulted in extra urinary homogentisic acid, corresponding to about 70% of the theoretical value. Similar results were obtained with patient R.T. This agrees with the observation of Mittelbach[6] that the conversion of the aromatic amino acids to homogentisic acid is more complete when small amounts are fed at short intervals.

It should be noted that the excretion of homogentisic acid derived from the ingested aromatic amino acid was rapid and was, with one exception (Series B, day 9), not prolonged beyond the experimental day. The prompt excretion after the administration of the phenylalanine is also shown in the elimination of homogentisic acid over shorter periods of time on the experimental days. Thus, in Series C, day 8, the H:N ratios were 212, 160, and 58 respectively for the urines collected over 6-, 6-, and 12-hour periods after administration of the phenylalanine. The very high ratio of 212 in the first 6-hour period indicates that the excretion of extra homogentisic acid began promptly after the ingestion of phenylalanine and the low ratio of 58 in the third period indicates that the extra excretion of the acid was nearly complete in the first 12 hours. Similar results were obtained in the other series during which the urine was collected over shorter periods of time than 24 hours.

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[3] Briggs, A. P., *J. Biol. Chem.*, 1922, **51**, 453.

[4] Garrod, A. E., *Inborn Errors of Metabolism*, Second Edition, 1923, 69.

[5] Plimmer, R. H. A., and Lowndes, J., *Biochem. J.*, 1937, **31**, 1751.

[6] Mittelbach, F., *Deut. Arch. klin. Med.*, 1903, **78**, 161.

Summary. Oral administration of 10 to 15 g daily of *l*(–)-phenylalanine to two alcaptonuric brothers resulted in a notable increase in the excretion of homogentisic acid in the urine, corresponding to about 70% of the amount theoretically obtained by complete conversion of the phenylalanine fed to homogentisic acid. The excretion of the extra homogentisic acid began promptly and was nearly complete within 12 hours after the phenylalanine was fed.

10003

A Mechanical Pump for the Drawing of Gas Samples.

S. B. BARKER AND EDWARD SMYTH. (Introduced by William H. Chambers.)

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Because of the interest aroused by a device made in this laboratory for the automatic drawing of samples from flowing gas, a brief description is being presented. The apparatus is in use here in conjunction with open-circuit respiratory metabolism determinations, the analyses for CO_2 and O_2 being performed on a Carpenter-Haldane analyzer,[1] but it can obviously be of service in any situation when it is desired to draw a continuous sample of gas over a set period of time.

Benedict and Ritzman[2] have described a method of drawing gas samples by attaching a Lee pump[3] to gearing activated by electromagnets controlled from the gas meter. The present apparatus uses a larger pump of similar construction but with the stopcock built into the cylinder head. The driving power is transmitted by gears to the threaded shaft from an electric motor which runs the blower. The sampler has the following qualifications:

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[1] Carpenter, T. M., in Abderhalden, E., *Handbuch der biologischen Arbeitsmethoden*, Berlin and Vienna, 1933, Abt. IV, Teil 13, 593.

[2] Benedict, F. G., and Ritzman, E. G., *Wissenschaft. Arch. f. Landwirtschaft*, 1931, Abt. B, **5**, 1.

[3] Lee, R. C., *J. Indus. Eng. Chem.*, Anal. Ed., 1933, **5**, 354.

Continuous drawing of a predetermined volume is obtained over a set length of time, not dependent upon the rate of gas flow. Since a single electric motor drives both the blower and the sampler through separate gearing, variations in gas flow due to fluctuations in electric current will automatically be compensated for in the aliquot drawn. The separate gearing allows the operator to vary the collection time of samples and the velocity of air passage independently of each other but insures a constant aliquot at all times.

Careful construction renders the sampler sufficiently gas tight so that a sample can be left in the apparatus (with the stopcock closed) for more than 12 hours with perfect safety.

Accurate estimation of the volume of samples can be obtained directly from the length of exposed pump shaft.

In all, a rugged system has been evolved, capable of functioning continuously over long periods of time without requiring constant adjustment and attention.

The original apparatus, built by Mr. James Evenden, has been giving satisfactory service for over 3 years;[4] several modifications suggested by experience with this have been incorporated in a newer design. This improved sampler, constructed by Mr. Smyth and Mr. R. E. Phipps, is diagrammed in Fig. 1, first in a horizontal cross-section through the middle and, below, in a rear view.

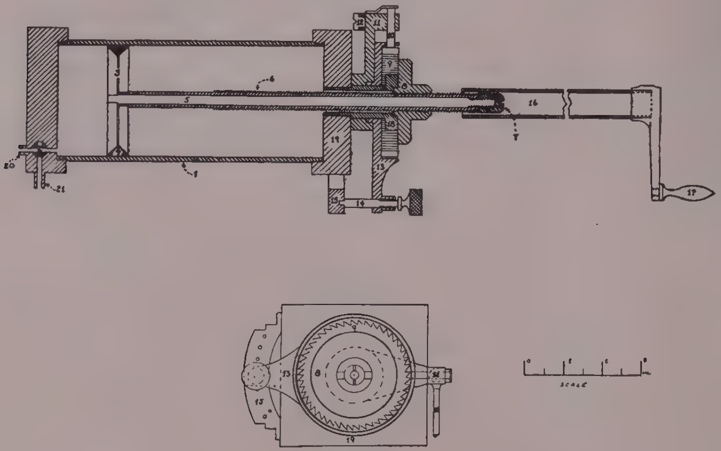

FIG. 1.

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[4] Barker, S. B., Chambers, W. H., and Dann, M., *J. Biol. Chem.*, 1937, **118**, 177.

The apparatus is essentially a hollow metal cylinder (*1*), into which gas is drawn through a small hole in a ground-in metal stopcock (*2*) by a mechanically operated plunger (*3*). In order to insure a tight fit for the plunger, the edges are sealed by 2 circular leather washers (*4*), triangular in cross-section, which together form a V-shaped wedge. It is absolutely necessary, as stressed by Lee,[3] to remove all fat from the rings and to relubricate with petroleum oil only, in order to avoid changes in the composition of the sample. The leather wedge fits between the 2 beveled halves of the plunger head (*3*) and can be expanded by drawing the two halves together. In order to make this adjustment without removing the plunger from the cylinder, the first plate is fastened to a solid shaft (*5*) which is inside the hollow shaft (*6*) connected with the other plate. By tightening a nut (*7*) on the threaded projecting end of the solid shaft, one squeezes the leather rings, producing a tighter fit. The apparatus should be tested for tightness on both reduced and increased pressure, especially the former, to rule out the possibility of sucking in gas from any place other than through the hole at the stopcock.

The outside of the hollow shaft (*6*) is threaded to fit an inside thread on a brass collar (*8*) fastened to a wheel (*9*) with teeth on its outer circumference. A bakelite ratchet tooth (*10*) turns the collar, by which action the plunger is pulled out. Rotary movement of the plunger is prevented by a key-way which remains engaged all the time. In order to prevent the piston from being drawn back too far in the cylinder, the threads on the appropriate portion of the shaft are stripped. When this area comes into the ratchet-wheel drive mechanism, the pulling motion stops. This is merely an emergency provision and, since a certain amount of careful manipulation is required to replace the shaft on the threads, the gearing should be so arranged as to permit maximum gas collection only up to the stripped area in the desired length of period.

The thrust motion of the arm (*11*) activating the ratchet can best be obtained by connecting the arm by a rod (*12*) to an eccentric driven by a motor. The length of collection period is determined by the number of teeth engaged in a single excursion of the ratchet. This is controlled by turning the guard (*13*) so that a certain number of teeth on the notched wheel are exposed in a slot. During the rest of its arc the ratchet rides on the guard, which is held firmly by a pin (*14*) set into a guide (*15*). At the end of the period the operator turns the guard so that the ratchet is entirely disengaged, thereby stopping the collection of gas. The metal stopcock (*2*) is turned to connect the sampler with a detachable gas storage pump, and the collected gas forced over by revolving the brass collar in the reverse direction to the one in which it was previously turning. For this purpose a long piece of hollow brass rod (*16*) with a crank (*17*) at the free end is slipped over the shaft, and two projections engaged in slots cut in the small portion of the collar (*8*). A bronze plug (*18*), pressed into the rear block (*19*), holds in place the rotating components of the gear-drive mechanism and also serves as a fulcrum against which the force of *8* and *9* can be exerted when the sample is being discharged.

In order to insure a tight, permanent connection between the sampler and the flowing gas, the projecting tip (*20*) is set into a washer in a hole in the pipe carrying the flow, a clamp drawing the pipe and the sampler firmly together. The other tip (*21*) is for connection through a short piece of heavy-walled rubber tubing to a detachable pump for storage and analysis. The detachable pumps have no gears, but have the same type of plunger and double shaft, which ends in a knob concealing the adjusting nut for the inner shaft. The capacity of these, as well as of the sampler, is about 700 cc. Excessive weight is avoided by employing duralumin wherever possible, except in the case of moving parts, for which brass is used as a dissimilar metal.

10004

The Excretion of Porphyrin in Pellagra.

KONRAD DOBRINER, W. H. STRAIN AND S. A. LOCALIO. (Introduced by C. P. Rhoads.)

From the Department of Medicine, University of Rochester School of Medicine and Dentistry, Rochester, N. Y., and the Hospital of the Rockefeller Institute for Medical Research, New York City.

Beckh, Ellinger and Spies[1] and Spies, Gross and Sasaki[2] have described an increased output of porphyrin in the urine in both endemic and alcoholic pellagra. A normal excretion of porphyrin was observed after the symptoms regressed following suitable treatment. In these experiments, isolated, small samples of urine were examined, determinations of the porphyrin content of the stools were not made, and the type of porphyrin excreted was not established. Hence it is not possible to conclude whether the porphyrinuria is due to an increase in the total output of porphyrin, or represents a normal total output with an increased proportion excreted by the liver through the urine. Furthermore, the lack of melting point determinations and quantitative studies makes it impossible to conclude whether the porphyria represents a simple increase of a normal process as shown by Dobriner and coworkers for orderly increased hematopoiesis, or a pathological metabolism of pigments marked by the excretion of coproporphyrin Type III.[3, 4]

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[1] Beckh, W., Ellinger, P., and Spies, T. D., *Quart. J. Med.*, New Series, 1937, **6**, 305.

[2] Spies, T. D., Gross, E. S., and Sasaki, Y., PROC. SOC. EXP. BIOL. AND MED., 1938, **38**, 178.

During a series of studies of the rate of excretion of porphyrin as influenced by the normal and pathological construction and destruction of hemoglobin it has been possible to make detailed observations in a case of pellagra. The rôle of the porphyrins in pellagra is of considerable importance, since the disease presents a certain similarity to porphyria in that both the conditions are characterized by a photosensitivity of the skin. Previous investigators, Massa[5] and Bassi[6] have suggested that the dermatitis of pellagra, like that of porphyria, is consequent to an increased content of porphyrin in the tissues.

Case Report. The patient, a 26-year-old colored woman, was admitted to the Strong Memorial Hospital, Rochester, New York, from May 11, 1936, to June 7, 1936, with a chief complaint of stomatitis, edema of the vulva, dermatitis, and diarrhea. Two months prior to admission the patient, a chronic alcohol addict who had been on a deficient diet for some time, noted mild stomatitis which became progessively worse. Shortly thereafter she developed dermatitis of the hands, feet, and elbows, and 2 weeks before admission the vulva became edematous and was associated with a foul smelling vaginal discharge and severe diarrhea.

Physical examination revealed typical symmetrical pellagrous dermatitis and stomatitis. The vulva and perineum were ulcerated and edematous. The remainder of the physical examination was irrelevant. The temperature was normal. Free hydrochloric acid was present in the gastric juice.

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[3] Dobriner, K., Strain, W. H., and Localio, S. A., PROC. SOC. EXP. BIOL. AND MED., 1937, **36**, 752.

[4] Dobriner, K., Rhoads, C. P., and Hummel, L. E., *J. Clin. Inv.*, 1938, **17**, 125, and earlier papers.

[5] Massa, M., *Riforma Medica*, 1932, **48**, 1669.

[6] Bassi, U., *Clin. Med. Ital.*, 1934, **65**, 241.

During the first 6 days of observation the patient was maintained on a fluid diet containing 2,000 calories daily, without clinical improvement. Following this control period, 75 g of a yeast extract (Vegex) were administered daily by mouth and 10.0 cc of liver extract intramuscularly (Eli Lilly and Co.) for 10 consecutive days. Improvement was noted on the second day of treatment and progressed rapidly. On the 8th day the lesions were greatly improved and the stools formed. Liver extract was discontinued on the 10th day and the Vegex was reduced to 50 g daily. During the 30 days of hospitalization the erythrocytes rose from 3.5 to 4.07 million, the hemoglobin from 11.2 to 12.2 g, the mean corpuscular volume fell from 102 to 92, and the reticulocytes reached their peak of 4% on the 16th day. Three months later the patient returned with a slight recurrence which responded rapidly to 4 injections of liver extract.

The methods used for quantitative determinations of the daily total excretion of porphyrin were similar to those previously described.[3, 7] The excretion rate of coproporphyrin is presented graphically in Fig. 1. During the 6-day control period the total coproporphyrin excretion averaged 897 mg per day; of this 254 were excreted in the urine. In the first 9 days of therapy coincidently with clinical improvement the total excretion fell to an average of 458 mg per day, of which 157 mg were in the urine. In the 2 following 9- and 6-day periods of observation the average total daily coproporphyrin excretion fell to 381 and 298 respectively. Besides the increased excretion of coproporphyrin, large amounts of protoporphyrin and deuteroporphyrin were found in the feces, a phenomenon which may have been consequent to bleeding into the intestinal canal.

The excreted coproporphyrin was a mixture of coproporphyrin I and coproporphyrin III[7] but since only small amounts of the Type III were excreted, sufficient material was not available for its final identification. Crystals of coproporphyrin I ester were obtained and were identified by their melting points (234°C). This mixed porphyrin excretion is similar to that reported for certain cases of porphyria, pigment cirrhosis of the liver, and lead poisoning and is different from that of pernicious anemia, hemolytic jaundice, and other diseases in which only increased coproporphyrin I and no coproporphyrin III is produced and excreted.[4, 7, 8–11]

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[7] Dobriner, K., *J. Biol. Chem.*, 1937, **120**, 115.

[8] Vigliani, E. C., and Libowitzky, H., *Klin. Wchnschr.*, 1937, **16**, 1243.

[9] Grotepass, W., *Z. f. physiol. Chem.*, 1932, **205**, 193.

[10] Watson, C. J., *J. Clin. Inv.*, 1937, **16**, 383, and earlier papers.

[11] Mertens, E., *Klin. Wchnschr.*, 1937, **16**, 61.

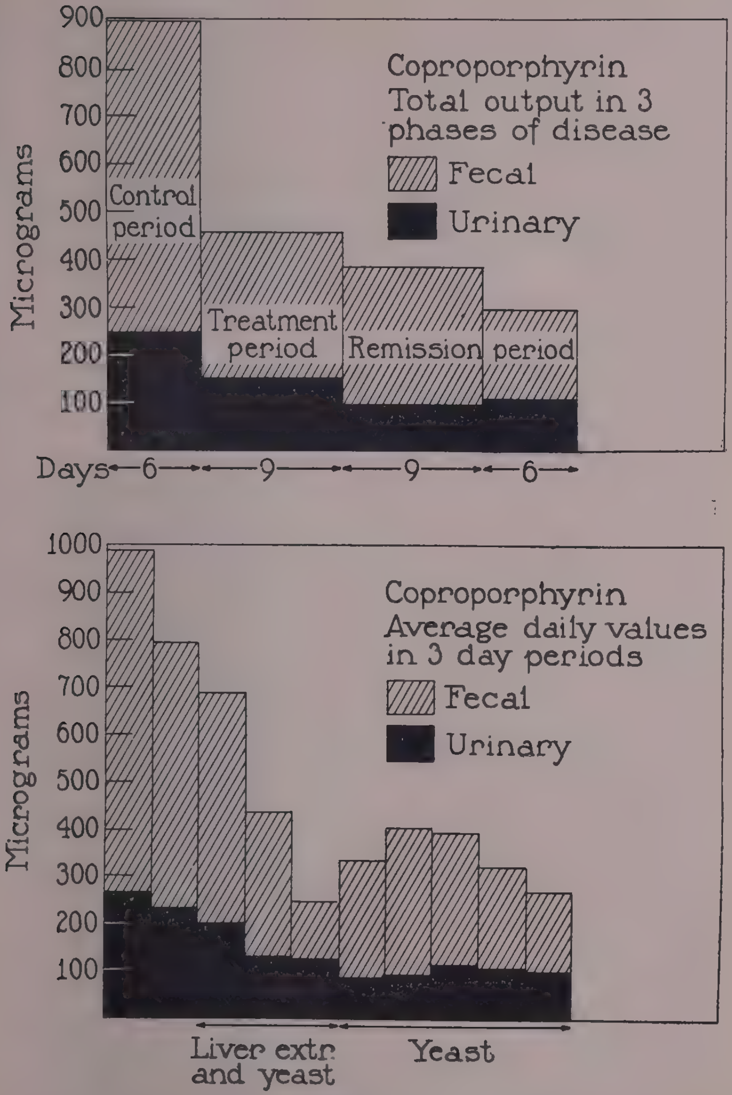

FIG. 1.

The fact that a pathologic construction as well as an abnormally great excretion of porphyrin has been demonstrated in a single case of alcoholic pellagra should be interpreted with care. Unpublished observations of material, furnished by Dr. Sydenstricker, from other cases of pellagra, do not favor the view that increased porphyrinuria is a constant feature of the disease. Furthermore, since porphyrinuria may indicate simply an hepatic insufficiency similar to that causing urobilinuria, the factor of liver dysfunction in pellagra warrants further study.

Summary. 1. Quantitative determinations of the excretion of porphyrin in a case of alcoholic pellagra are presented. 2. Both quantitatively and qualitatively abnormal excretion of porphyrin featured relapse and disappeared during the induced remission.

10005

Natural Meningococcal Agglutinins and Lysins in Human Serum.

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The study of the susceptibility-problem in cerebrospinal fever, a disease characterized by a highly selective incidence, has divided itself naturally into an analysis of the rôle played by the causative agent and the factors concerned with host-resistance. Investigations dealing with the organisms have centered chiefly around the carrier-condition as a source of infection and the significance of the serological types of meningococci; those dealing with the host have been mostly concerned with attempts to evaluate the humoral defences of the body against meningitic infection, it being unknown what causes the selective localization of the invading organisms in the meninges.

Together with phagocytosis, the bacteriolysin and agglutinin of human blood are regarded by some authors as the primary agents concerned with the removal of the meningococcus from the circulation following its initial invasion.[1, 2] It is generally accepted that not only the normal serum of man but also that of a number of animals (guinea pig, rabbit and monkey) are strongly meningococcidal *in vitro*.[3, 4] The normal bacteriolysin resembles other natural antibodies in that its titer is said to increase with age in man and animals, regardless of previously known exposure to the meningococcus; Silverthorne and Fraser,[5] however, stress the strong menin-

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[1] Murray, E. G. D., Medical Res. Council, spec. Rep't Series No. 124, 1929.

[2] Heist, S., Solis-Cohen, S., Solis-Cohen, M., *J. Immunol.*, 1922, **7**, 1.

[3] Flexner, S., *J. Exp. Med.*, 1907, **9**, 105.

[4] Matsunami, T., and Kolmer, J., *J. Immunol.*, 1918, **3**, 177.

[5] Silverthorne, N., and Fraser, D. T., *J. Immunol.*, 1935, **29**, 523.

gococcidal power of the blood of carriers as indicating its specific character. The same uncertainty prevails regarding the nature of the natural agglutinin which has variously been reported as being present or absent in the blood of normal persons, carriers, or convalescents.[6, 7, 8]

In view of the discrepancies cited above a study of normal human sera was undertaken in order to investigate not only the frequency of the occurrence of both lysin and agglutinin, but also to determine the relationship between the two antibodies and their specificity.

Methods. Three strains of meningococcus were used in this work: 2 of these represented stock strains of groups I-III and II-IV respectively, the third strain was freshly isolated from the nasopharynx of a healthy carrier and belonged to group II-IV. The human sera were used either unheated or heated to 60°C for ½ hour. The donors of these sera ranged in age from 14-72 years and gave no history of either a previous attack of meningitis or of unusual conditions of the upper respiratory tract. The agglutination-tests were set up with a variety of antigens, including suspensions of live cocci and of organisms killed by either formalin or phenol. The results were recorded after incubation at 37° for 2 hours and again after leaving the tubes in the icebox overnight. The lysin was determined by mixing 0.02 cc of undiluted serum plus 0.2 unit of guinea-pig alexin with 0.02 cc of dilutions of 1:1000, 1:10,000, and 1:100,000 of a freshly prepared heavy suspension of live cocci. After incubation for 24 hours, smears were made and examined microscopically for the presence of intact meningococci. This procedure gave results identical with the plating out of the serum-meningococcus mixtures and counting of developing colonies.

Results of the Agglutinative tests. 141 adult human sera and 26 sera from infants or children (ages 1 month to 9 years) were tested for agglutinins against the 3 strains of meningococcus representing the 2 broad serological groups. The results are given in Table I. It is apparent that about one-half of the adult sera agglutinated group I-III meningococcus whereas almost two-thirds of these sera contained agglutinins against group III-IV organisms. Further analysis of the individual reaction of each serum yielded the following data: 48 sera (34%) lacked agglutinins for both strains while 49 sera

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[6] Stoevesandt, K., *Cent. f. Bakt. orig.*, 1908, **46**, 295.

[7] Gates, F. L., *J. Exp. Med.*, 1918, **28**, 449

[8] Worster-Drought, C., and Kennedy, A. C., *Cerebrospinal Fever*, London, A. and C. Black, Ltd., 1919.

TABLE I.

Incidence of Natural Meningococcal Agglutinins and Lysins in Normal Human Serum.

	Meningococcus I-III				Meningococcus II-IV					Meningococcus I-III				Meningococcus II-IV			
Agglutinin Titer*	Adult Sera		Infants' and Child. Sera		Adult Sera		Infants' and Child. Sera		Lysin Titer†	Adult Sera		Infants' and Child. Sera		Adult Sera		Infants' and Child. Sera	
1:160	32		0		36		0		Undiluted	0		0		0		0	
1:80	14		0		8		0		1:1,000	3	81(92%)	0	6(18%)	1	81(92%)	0	6(18%)
1:40	7	69(49%)	0	1(3.8%)	14	91(64.6%)	1	5(19.2%)	1:10,000	64		6		59		6	
1:20	5		0		10		1		1:100,000	14		0		21		0	
1:10	7		1		12		2		0	7	(8%)	28	(82%)	7	(8%)	28	(82%)
1:5	4		0		11		1										
0	72	(51%)	25	(96.2%)	50	(35.4%)	21	(80.8%)	Total	88		34		88		34	
Total	141		26		141		26										

*Indicates highest dilution of serum producing distinct clumping.

†Indicates lowest dilution of bacterial suspension lysed by serum.

(35%) agglutinated both strains; 11 sera (7.8%) agglutinated I-III strains only and 33 sera (23%) agglutinated II-IV strains only. It is also clear that organisms belonging to group II-IV were agglutinated, almost uniformly, to higher titers. In contrast to these results, only 3.8 and 19.2% of the sera from infants and children showed agglutinins of a low titer against groups I-III and II-IV meningococci, respectively. The agglutinin is evidently heat-stabile since heating for ½ hour at 60°C did not appreciably change the titer of the serum.

Results of the Bacteriolytic Tests. 88 normal adult sera and 34 sera from infants and children (ages ranging from 12 days to 4 years) were tested for lytic activity against the 2 serological groups of meningococci. As may be seen from Table I, fully 92% of the adult sera showed some bacteriolytic effect against either group of meningococci, no sera being lytic for one strain and not for the other. Moreover, the end-titers were approximately the same for both groups of organisms. On the other hand, 82% of the infants' and children's sera were devoid of meningococcidal power under the conditions of our test. The bacteriolytic complex requires "complementation" as shown by the absence of lysis when heated sera were brought together with cocci without the addition of alexin.

Relationship Between Agglutinin and Lysin. As regards the coexistence of the natural agglutinin and lysin in the same serum, our data indicate that 19 of 95 lytic sera—20% of all sera examined for the simultaneous presence of the 2 antibodies—failed to agglutinate either serological group of cocci. However, in no case was the reverse encountered, *i. e.*, absence of lysin in an agglutinating serum. Again, if one compares the agglutinative titer with the lytic strength against any one group of meningococci, no quantitative relationship becomes evident as indicated by the fact that a given serum may agglutinate only feebly but be markedly lytic, and *vice versa*.

Specificity of the Natural Agglutinin and Lysin. Experiments to determine the degree of immunological specificity of the 2 antibodies were carried out by absorbing pooled normal adult serum with homologous and heterologous organisms and testing the sera after absorption for agglutination and lysis of the various members of the genus *Neisseria*. The results indicated that the natural meningococcal agglutinin possesses well marked type- and species-specificity, only absorption with the homologous type meningococci causing complete removal of the respective agglutinins. The same experiment, however, demonstrated a complete lack of immunological specificity for the natural meningococcal lysin, as evidenced by the fact that this substance may be removed not only by absorption with the heterologous type meningococci but by contact with *B. coli* as well. In a similar absorptive test in which specific rabbit-anti-meningococcal serum was substituted for normal human serum, both the immune agglutinin and lysin exhibited a high degree of immunological specificity, contact with *B. coli* causing no change in the agglutinative titer and only a minor diminution of the lytic activity.

Animal Sera. A small number of normal sera from various animals, including the monkey, rabbit, guinea pig and rat were examined for their content of natural meningococcus agglutinins and lysins. Suffice it to state that all sera tested showed marked meningococcidal power with no or negligible agglutinin titers.

Conclusions. Our data permit certain deductions regarding the nature and origin of the natural meningococcal agglutinin and lysin. It is clear that both antibodies increase in intensity and extensity with age and since conditions are generally favorable for the successive absorption from the nasopharynx of antigenic material belonging to the genus *Neisseria* one might be led to believe that both are due to "occult" immunization. However, as will appear below, it is more than likely that we are dealing with two separate entities.

The natural meningococcal agglutinin, according to our data, possesses a well marked immunological specificity. This is suggested not only by the occurrence of type-specific agglutinins in a certain percentage of normal human sera but more particularly by the fact that such agglutinins can be exhausted only by absorption with the specific antigens. In this respect the natural agglutinin resembles the immune agglutinin. Our observation that natural agglutinins occurred more often against group II-IV than against group I-III strains of meningococci is further suggestive evidence in favor of assuming their specific origin inasmuch as Branham[9] had found a preponderance of II-IV strains among cultures obtained from carriers whereas a majority of cultures isolated from cases of meningitis belonged to group I-III. In contrast to what has been said about the natural agglutinin, our data show that the power of normal human serum to lyse the meningococcus is invariably directed against both serological groups, and absorption-experiments revealed a complete lack of immunological specificity. The natural meningococcal lysin, therefore, appears to be quite different from the marked increase in meningococcidal power which is obtained following immunization with the specific organisms. This is also borne out by the fact that the lytic property in normal human serum may exist entirely apart from its agglutinating capacity. The conclusion, therefore, seems justified that the normal meningococcidal power of human and animal blood is wholly unrelated to any previous immunizing experience with the specific antigen. While the possibility cannot be excluded that in those instances in which natural agglutinin and lysin occur together both antibodies reflect the result of common antigenic stimulation, it is more than likely that this relationship is merely fortuitous and that their coexistence should be interpreted as a chance occurrence of 2 different substances, one a specific antibody and the other a nonspecific factor of the blood.

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[9] Branham, S., *J. Am. Med. Assn.*, 1937, **108**, 692.

10006

Citrate Solutions for Preservation of Fluid Blood.

JOYCE COTTER AND W. J. MACNEAL.

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Solutions of sodium citrate have been used to prevent coagulation of blood for many years and these solutions have become increasingly important in preserving the fluid blood for transfer from one human being to another. The citrate solutions are also employed in various laboratory procedures, particularly in the preservation of leukocytes for study of phagocytosis. The present study was undertaken because of the suspicion that some untoward reactions observed following citrate transfusions might be related to the citrate.

We obtained hermetically sealed ampules of the citrate solutions of 4 different manufacturers. All of these were perfectly clear. A test of the hydrogen-ion concentration of these 4 specimens revealed the following results:

Specimen	pH
A	8.5
B	8.0
C	8.1
D	8.2

We also prepared some solutions in our own laboratory and found that one solution of sodium citrate, 2.5% in 0.6% sodium chloride, had a pH of 8.7, higher than that observed in any of the 4 commercial samples.

We prepared a solution of citric acid, 2.5% in 0.6% sodium chloride, and found that this solution of citric acid had a pH of 1.17. A number of trial mixtures of citric acid and sodium citrate were then prepared and it was found that a mixture of 5 cc of the citrate with 0.03 cc of the citric acid gave an approximately neutral solution. The various solutions were then tested by mixing 1 cc of freshly drawn human blood with 4 cc of the undiluted citrate solution. These mixtures were incubated at 37°C for 20 hours, then placed in the refrigerator for 24 hours, and then left in the room for a week. The appearance of the preparation was recorded from time to time. At the end of a week there was very marked destruction of the red blood corpuscles with red discoloration of the supernatant liquid in those tubes in which the citrate solution of hydrogen-ion concentration 8.5, 8.1, and 8.2 had been used. In the citrate of pH 8.0 the hemolysis was distinctly less.

Without going into great detail in regard to the many tests which were performed, one may say that the blood, when mixed with citrate solution of pH 7.1 to 7.5, remained for many days, even at room temperature, without evidence of dissolution of the red blood corpuscles. When, however, the citrate solution had a hydrogen-ion concentration greater than 7.6 or less than 6.8, one observed gross evidence of destruction of the blood.

A further problem presented itself in regard to sterilization of these citrate solutions in glass ampules. It is evident that the quality of the glass plays a part in determining the final results. One should, therefore, work with glassware of uniform composition and then will be able, by trial and error, to determine the exact mixture of citric acid and citrate which will, after proper sterilization in the autoclave, retain the desired hydrogen-ion concentration.

With our materials it was found best to add 0.04 cc of 2½% citric acid solution to 5 cc of the 2½% sodium citrate solution, in order to obtain, after autoclave sterilization, a solution with a pH between 7.1 and 7.5. When fresh human blood was mixed with such a solution without it being diluted with saline, the blood corpuscles remained without evidence of hemolysis for at least a week at room temperature.

There is every reason to expect that such a solution diluted further with saline, which is itself neutral in reaction or nearly so, will permit the preservation of blood at low temperatures for many days or weeks. Because of the increasing interest in the use of citrated blood in transfusion, these observations may have some practical value.

10007

Neutralization of Ovarian Follicular Hormone in Women by Simultaneous Administration of Male Sex Hormone.*

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Recent experiments from this laboratory, in which the vaginal smear was used as an index, have shown that the menstrual cycle can be inhibited in the human by the male sex hormone.[1] This is in accord with previous findings in rodents[2] and primates.[3] The vaginal smear reverts to the atrophic or menopausal type,[4] and can be kept at that level by continued administration of adequate amounts of hormone. Menstrual function returns spontaneously after cessation of treatment.

The present study was concerned with the possibility that the male hormone might have a direct antagonistic action on the ovarian follicular hormone, in addition to its suppressive effect on menstrual function. It was stimulated by our findings in a case in which the menstrual cycle was interrupted by the administration of testosterone propionate. Four weeks after the last definitely established ovulatory phase, and during a period in which the vaginal smear remained typically menopausal, there was observed an increased excretion of ovarian follicular hormone which reached the same high levels as during the previous follicular phase, and lasted for 3 days. The question arose whether the persistence of the menopausal smear during this period might have been due to neutralization of the effect of endogenous estrogen on the vaginal epithelium by the administered male hormone. The experiments of Robson[5] on ovariectomized mice in which vaginal cornification by oestrone was prevented by concomitant administration of testosterone, suggested that this might be the case.

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* Aided by Grants from the Josiah Macy, Jr., Foundation and from the Committee for Research on Problems of Sex, of the National Research Council.

[1] Papanicolaou, G. N., Ripley, H. S., and Shorr, E., Proc. Soc. Exp. Biol. and Med., 1938, **37**, 689.

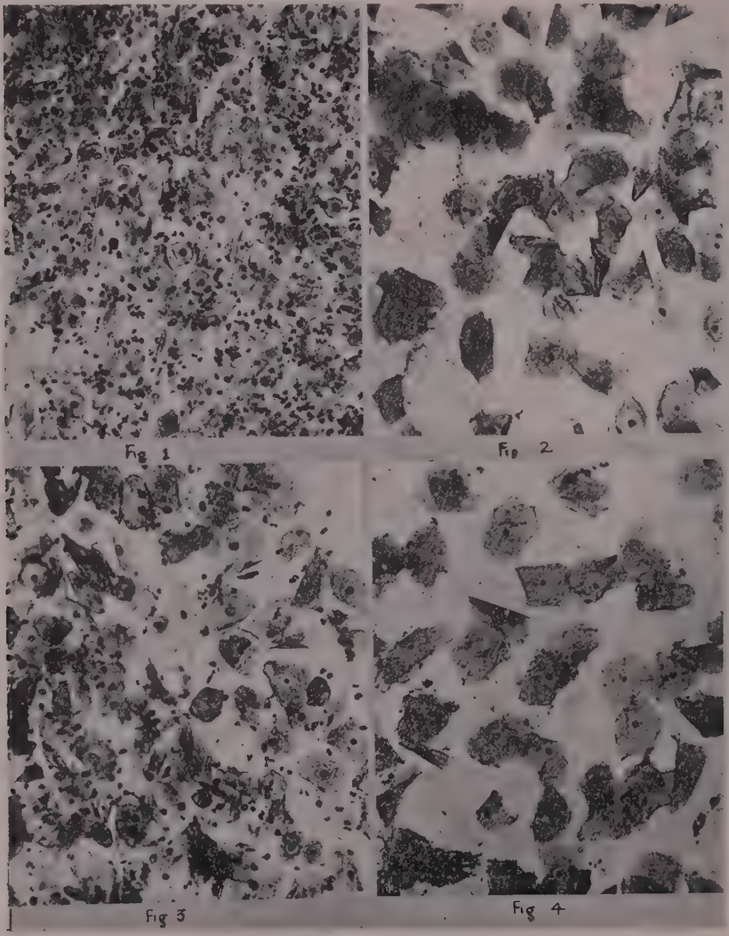
[2] Robson, J. M., Proc. Soc. Exp. Biol. and Med., 1936, **35**, 49.

[3] Zuckerman, S., *Lancet*, 1937, **233**, 676; Hartman, C. G., Proc. Soc. Exp. Biol. and Med., 1937, **37**, 87.

[4] Papanicolaou, G. N., and Shorr, E., *Am. J. Obs. and Gyn.*, 1936, **31**, 806.

[5] Robson, J. M., *J. Physiol.*, 1937, **90**, Proc. Physiol. Soc., 15 P.

Experimental. With this in mind, estradiol benzoate (progynon-B) was administered to women with the menopausal syndrome in amounts sufficient to produce and maintain a follicular smear.[4] At



Vaginal Smears of Case C.

FIG. 1. Control smear before treatment (menopausal type).

FIG. 2. Follicular smear following treatment with estradiol benzoate (3,000 R.U. daily), prior to administration of testosterone propionate.

FIG. 3. Smear after daily administration of 25 mg of testosterone propionate for 27 consecutive days and 50 mg for 7 days, combined with 3000 R.U. of estradiol benzoate daily throughout this period. Note disappearance of cornified cells, reappearance of deep cells, with regression towards the menopausal type of smear.

FIG. 4. Reappearance of follicular smear with continuance of daily treatment with 3000 R.U. of estradiol benzoate, 14 days after stopping testosterone propionate.

this stage, the injections of estradiol were continued, and, at the same time, testosterone propionate (Oreton)† was also given. The effects of the simultaneous administration of these hormones was followed by means of vaginal smears, vaginal biopsies, and hormonal assays.

The subjects were 3 women whose menopause was well established. Patients A and B had intact pelvic organs. Patient C had had a hysterectomy. In all 3, control smears were typically menopausal (Fig. 1). Prior to treatment, urinary estrogen assays in each case indicated a daily excretion of less than 90 International units, and the urinary prolan was above normal levels.

A daily dose of estradiol benzoate sufficient to establish a typical follicular smear (Fig. 2) and symptomatic relief was administered. This was, for A, 1,500 R.U., and for B and C, 3,000 R.U. Then testosterone propionate was given as follows: to A, 25 mg daily in oil for 17 days (total 425 mg); to B, 25 mg daily for 10 days, 50 mg for 3 days, and 25 mg for the next 3 (total 475 mg); and to C 25 mg on 27 consecutive days, and 50 for the following 7 (total 1,025 mg). Estradiol benzoate, in the doses given above, was administered throughout the period of study.

In all 3 women, with the administration of male hormone together with estradiol benzoate, there was a gradual disappearance of cornified cells, a reappearance of deep cells, and the smears lost their typical follicular character (Fig. 3). Biopsies of the vaginal mucosa at this time showed a loss of the cornified zone which had been built up by the previous injections of estradiol benzoate. The level of the urinary prolan fell to below 10 mouse units per 24 hr and remained within these normal values throughout the period of combined treatment. The symptomatic improvement achieved with estradiol alone persisted when both hormones were given. During the period in which the male hormone was given, libido and sexual response were definitely greater than that experienced with estradiol alone. The voice was perceptibly lowered, and there was a marked vulval hyperemia, as well as moderate enlargement of the clitoris in all 3 subjects.

In B and C, the administration of estradiol benzoate was continued after the testosterone propionate was stopped, and the vaginal smears again became follicular (Fig. 4). Patient C received a second course of 25 mg of testosterone daily for 14 days, along with

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† We are indebted to Dr. E. Schwenk and Dr. G. Stragnell of the Schering Corporation for the generous supply of testosterone propionate (Oreton) and estradiol benzoate (Progynon B).

3,000 R.U. of estradiol benzoate. This resulted in a second transition from a follicular smear to one devoid of cornified cells.

A second group of 5 menopausal women, including 2 castrates, received testosterone propionate alone (25 mg daily). Symptoms were relieved with this dose in 4, and prolan excretion diminished, with no changes in the smear picture or the vaginal epithelium, which remained menopausal throughout. In neither of these 2 groups of subjects was there observed the development of a follicular smear with male hormone alone as has been recently reported by Salmon.[6]

Comment. There are several possible modes of action of male hormone in women. One is a direct effect upon the ovary to suppress menstrual function. An example of this may be the amenorrhea which follows the prolonged administration of the hormone to menstruating women.[1] Another possibility is that the action is primarily on other endocrine organs, most likely the hypophysis, and the effect on the ovary is secondary. This is suggested by the diminution in prolan excretion we and others[6] have observed. A third is a direct peripheral effect antagonistic to the estrogens, such as we have shown for vaginal cornification in humans, and Robson in mice. Finally, all these mechanisms may function in varying degrees and at different times.

A calculation of the neutralizing capacity in humans of the male hormone on estradiol is of interest. One mg of estradiol benzoate is equivalent to about 6,000 R.U. In these experiments, 25 mg of testosterone propionate was able to neutralize 0.5 mg of estradiol. The ratio is therefore 50:1. Using ovariectomized mice, Robson found a ratio of 300:1 between testosterone and estradiol.

These results may have some bearing on the interpretation of the observation that androgens and estrogens are both present in the normal male and female. It may be that some such mechanism of neutralization serves to preserve the fundamental characteristics of each sex, in the presence of both types of hormones, and that the different concentration in which they exist in men and women is the expression of the proper balance between them which will achieve this end.

Conclusion. Studies of vaginal smears and biopsies indicate that the male hormone (testosterone propionate) is able to neutralize, in women, the effects of estradiol on the cornification of the vaginal epithelium.

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[6] Salmon, U. J., PROC. SOC. EXP. BIOL. AND MED., 1937, **37**, 488.

10008

Content in Spreading Factor and Toxins in Organs and Poisonous Secretions of Snakes.

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While moderate amounts of the active spreading factor exist in practically all mammalian tissues[1] as well as those from other zoological groups* the most active products so far described have been associated with cell secretions. These may be noted as follows: (a) secretions endowed with a powerful physiological effect such as those from the testicle of mammals,[3] (b) secretions having anticoagulating powers as that from the leech,[4] (c) toxic secretions exemplified by the venom of certain snakes,[5] (d) soluble substances or secretions from toxic and invasive bacteria (Staphylococcus, Streptococcus, etc.).[6]

The idea suggested by these facts is that the living cells may be only a potential source of the factor, the activity of which does not become manifest until the secretion itself has undergone some change.† With this deduction in mind the secretions of certain poisonous snakes have been tested for their content of spreading and local toxic effect, in comparison with similar properties of extracts of the secreting glands.

Six different species of snakes were used, 4 poisonous and 2 nonpoisonous. The potency of the spreading factor in venom, blood serum and tissue extracts of these snakes was determined. The tissues were ground and extracted with 1 or 10 volumes of saline and the resultant pulps were centrifuged. From the supernatant fluids as well as from the blood serum and the venom, progressive dilutions were made in saline. In 3 of the experiments dealing with poisonous snakes the venom gland was cut with scissors and thoroughly washed with saline in order to remove all traces of secreted venom.

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[1] Duran-Reynals, F., *J. Exp. Med.*, 1929, **50**, 327; Claude, A., and Duran-Reynals, F., *J. Exp. Med.*, 1934, **60**, 457.

* Moderate amounts of spreading factor have been demonstrated in several species of birds, fish, lizards, newts, frogs, toads, snails, and harmless insects.[2, 5]

[2] Duran-Reynals, F., unpublished observations.

[3] Duran-Reynals, F., *C. R. Soc. Biol.*, 1928, **99**, 6; *J. Exp. Med.*, 1929, **50**, 327; Hoffman, D. C., and Duran-Reynals, F., *J. Exp. Med.*, 1931, **53**, 387; McClean, D., *J. Path. and Bact.*, 1931, **34**, 459.

[4] Claude, A., *J. Exp. Med.*, 1937, **66**, 353.

[5] Duran-Reynals, F., *Science*, 1936, **83**, 286; *J. Exp. Med.*, in press.

[6] Duran-Reynals, F., *J. Exp. Med.*, 1933, **58**, 161; McClean, D., *J. Path. and Bact.*, 1936, **42**, 477.

† The supposition outlined above is supported by experiments that indicate that some tissues are potential sources of large amounts of spreading factor. This factor may be liberated under certain conditions such as autolysis (*in vitro*) of rabbit's brain,[2] experimental intestinal obstruction in the same animal,[2] and certain bacterial infections.[2] The malignant transformation of a tissue also results in a marked increase of spreading factor.[7, 8]

[7] Duran-Reynals, F., and Stewart, F. W., *Am. J. Cancer*, 1931, **15**, 2790.

[8] Boyland, E., and McClean, D., *J. Path. and Bact.*, 1935, **41**, 553.

Two parts of each dilution were mixed with one part of India ink diluted 1:2, and 0.75 cc of each of the mixtures were injected intradermally in one side of rabbits. In these injections the India ink served as an indicator of the area of spreading of the mixtures. The same injections were repeated in the other side of the same animal using saline solution in place of the India ink, in order to be able to judge the severity of the resultant lesions uncomplicated by the presence of the ink. A mixture of 0.50 cc of saline plus 0.25 cc of ink was always injected as a control. Results after 24 hours are recorded in Table I.

The results shown in Table I seem to warrant the conclusion that the spreading and toxic power of extracts of the poison gland are much lower than the spreading and toxic power of its secretion, the venom. This difference is still more marked when the gland has been cleansed of any secreted venom adherent to its tissue. The fact that washing removes much of the toxicity suggests that traces of venom still remaining in the excretory ducts are responsible for at least part of the toxicity still shown by gland extract. Extracts of the poison gland at 1:1 dilution have a toxic and spreading effect only slightly superior to that shown by dilutions of the venom at 1:1000. The content of spreading factor of the poison gland does not seem to be greater than that found in other tissues of the snake.

The results show that there is a close correlation between the strength of the spreading factor and the toxicity of the venom or extracts of the poison gland. This is in line with our previous observation[3] which was that both spreading and toxic power of the venom are neutralized by the specific antiserum. The present findings suggest that both spreading factor and toxicity are a product rather than a component of the gland cell.

The table also shows that the spreading factor exists in moderate amount in the extracts of most tissues from both poisonous and non-poisonous snakes. Testes, from snakes yield no more of the factor than the other tissues which is contrary to the findings in mammals.

TABLE I.

Content in Spreading Factor and Toxin in Salivary Secretion and Tissues of Poisonous and Nonpoisonous Snakes Tested by Intradermal Injections in the Rabbit.

	Areas of spread and lesions produced by injection of .50 cc tissue extr. of venom + .25 cc India ink or saline sol. Dilution injected				Area of spread of .50 cc of saline + .25 cc India ink
	1:1 cm^2	1:10 cm^2	1:100 cm^2	1:1000 cm^2	
Rattlesnake poison gland 1st Exp., not washed		52.8 mod. severe	41.1 mild	6.5 no lesion	6.7
Same washed, 2nd Exp.	35.5 mild	14.1 very mild	10.0 very mild	8.1 ” ”	6.9
Same washed 3rd Exp.	39.0 ”	15.2 ” ”	11.6 ” ”	9.6 ” ”	7.8
Water moccasin poison gland, washed	20 mod. severe	7.1 mild	7.0 mild	8.2 very mild	5.8
Pine snake supralabial gland		12.9 no lesion	5.5 no lesion	5.1 no lesion	5.0
Chicken snake supralabial gland		30.0 ” ”	10.0 ” ”	6.0 ” ”	5.0
Pine snake ovary		6.1 ” ”	6.0 ” ”	6.0 ” ”	6.1
” ” spleen		15.5 ” ”	8.6 ” ”	4.5 ” ”	4.5
” ” muscle		4.7 ” ”	4.5 ” ”	4.0 ” ”	4.2
Rattlesnake, chicken snake testicle (avg)		8.5 ” ”	6.0 ” ”	6.1 ” ”	6.1
Rattlesnake, chicken snake, and pine snake liver (avg)		18.5 ” ”	7.5 ” ”	6.1 ” ”	6.0
Rattlesnake, chicken snake, and pine snake pancreas (avg)		14.0 mild	8.8 ” ”	5.8 ” ”	5.8
Rattlesnake, chicken snake, and pine snake blood serum (avg)		10.5 ”	6.8 ” ”	6.2 ” ”	5.8
Rattlesnake, chicken snake, and pine snake kidney (avg)		12.1 no lesion	9.7 ” ”	5.8 ” ”	5.8
Water moccasin venom		90.0 very severe	45.0 severe	15.1 mild	7.6
Rattlesnake venom (avg 6 tests)		150 ext. severe	95.0 very severe	30 mod. severe	6.3

On the other hand blood serum of snake shows some activity while that of mammals is practically inactive.

Summary. Spreading factor and venom are found only in negligible amounts in the secreting cells of the venom gland of snakes. As both the factors appear together in the secretion they either act together as a unit or are the same substance. The spreading factor in moderate amounts exists in most tissues from both poisonous and harmless snakes.

10009 P

Cerebral Mechanisms in Auditory Localization.*

EDWARD GIRDEN. (Introduced by Carney Landis.)
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Bikov[1] has reported that following complete transection of the corpus callosum it was possible to condition a dog to salivate to the sound of a whistle placed at the level of, and some distance from, the left ear. He was unable to subsequently develop a discrimination so that the animal did not salivate to the same stimulus sounded from its right side. Pavlov[2] concluded from this study that "a differentiation of the direction of a sound required a united activity of both hemispheres."

Bikov's data and Pavlov's interpretation are consistent with the view that hearing is crossed. That is, impulses produced in the left cochlea end in the right hemisphere, while those impulses originating in the right cochlea terminate in the left hemisphere. Since both ears are essential for the correct localization of sounds in space (L-R habit), destruction of the corpus callosum would prevent the integration of the impulses produced in the peripheral mechanisms. From this logic, it would also follow that extirpation of the auditory cortex of one hemisphere (temporal lobe) would have the same effect as does the destruction of one cochlea.

Recent studies, however, indicate that this view is incorrect. Results procured in the dog[3] and the cat[4] support the contention that

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* This study was made possible by grants from the American Philosophical Society and the Elizabeth Thompson Science Fund.

[1] Bikov, K. M., and Speransky, A. D., *Col. Papers, Physiol. Labs. of I. P. Pavlov*, 1924, **1**, 150.

[2] Pavlov, I. P., *Conditioned Reflexes*, Oxford University Press, 1927, p. 150.

[3] Mettler, F. A., Finch, G., Girden, E., and Culler, E., *Brain*, 1934, **57**, 475.

[4] Brogden, W. J., Girden, E., Mettler, F. A., and Culler, E. A., *Am. J. Physiol.*, 1936, **116**, 252.

hearing involves uncrossed as well as crossed components. Impulses from each cochlea reach both cortices. There is also some question as to the reliability of the salivary conditioning technic when it is employed in studies involving cerebral extirpation. Zeliony,[5] using this method failed to establish conditioned reflexes in a totally decorticate dog, whereas positive results have been reported both by Culler[6] and Girden[7] who made use of the motor conditioning technic. In the light of these facts, a retest of the question was made with the latter method. The L-R habit[8] was established by training the dogs to flex a forepaw upon the presentation of an auditory stimulus (buzzer or pure tone) from its right side, but to inhibit this response if the stimulus was sounded from the left side. All surgical operations were made under aseptic conditions and general, injection, anesthesia.

Although the histological examination of the brains is now in progress, the following results are clearly indicated. The L-R habit persists following complete transection of the corpus callosum, removal of the temporal lobe, with varying amounts of adjacent occipital and parietal tissue, of one hemisphere. The L-R habit is disrupted only if both temporal lobes are extirpated. If the 2 auditory areas are removed in successive stages (one month apart), the auditory conditioned reflexes still persist; the dog will still flex its paw to the auditory stimulus, regardless of its locus in space. If the bitemporal operation is performed in one stage, then not only is the L-R habit disrupted but all auditory conditioned reflexes disappear. The cortical area essential for the L-R habit lies within the limits of the sylvian gyrus (78-78b[9]).

These results, contrary to the Pavlovian interpretation, are in agreement with the more recently established beliefs concerning the distribution of the auditory pathways in the brain. Support is given to the contention that the motor conditioning technic is more reliable than the salivary method in studies which involve cerebral extirpations. The investigation is now being extended to determine what part of the sylvian gyrus is essential for the L-R habit, and whether particular areas within it function in the perception of specific frequencies or tones.

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[5] Zeliony, G., *Trans. Soc. Rus. Phy. of St. Petersburg*, 1911-12, **50**, 147.

[6] Culler, E., and Mettler, F. A., *J. Comp. Psychol.*, 1934, **18**, 291.

[7] Girden, E., Mettler, F. A., Finch, G., and Culler, E., *J. Comp. Psych.*, 1936, **21**, 367.

[8] Girden, E., *Am. J. Psych.*, in press.

[9] Papez, J. W., *Comparative Neurology*, Crowell, 1929.

10010

Prophylactic and Therapeutic Effect of Sulfonamide Compounds in Experimental Malaria.

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The treatment of malaria, either in man or experimental animals, with any of the accepted antimalarial drugs is not entirely satisfactory. A high percentage of relapses occurs in spite of vigorous therapeutic measures. In view of this and many other recognized deficiencies, it seemed worth while to try the effect of sulfonamide compounds on controlled experimental malarial infections. The reports in the literature concerning the chemotherapeutic action of the sulfonamide compounds on malaria are very meager. De Leon[1] and Hill and Goodwin[2] used prontosil in acute vivax malaria, and reported beneficial results, while Read and Pino[3] employed sulfanilamide and stated that it was without value. Van der Wielen[4] has also reported the use of prontosil in 2 cases of quartan malaria with a favorable therapeutic result.

The following report concerns the prophylactic and therapeutic effect of 2 sulfonamide compounds, sulfanilamide* (paramido benzene sulfonamide) and sulfanilyl sulfanilate,† in experimental malaria and the action of these drugs on the parasites *in vitro*. Both preparations were injected intraperitoneally and the former was also given by mouth.

Experiment 1. Two rhesus monkeys, Nos. 1 and 2, were each inoculated intraperitoneally with one million *Plasmodium knowlesi* parasites. Monkey 1 was then given 0.25 g of sulfanilamide intraperitoneally, and the same amount of the drug was given on each of 4 successive days thereafter. Monkey 2 served as a control. No infection developed in test Monkey 1 while control Monkey 2 became infected and died 12 days after inoculation.

Experiment 2. Inasmuch as the parasites and the first dose of sulfanilamide were injected simultaneously into the abdominal cavity, a direct parasiticidal effect was considered possible. In order to rule out this possibility, 2 monkeys, Nos. 3 and 4, were each inoculated with one million parasites, but treatment was withheld in the test animal until parasites appeared in the blood stream. Circulating parasites were first detected in both animals on the 4th day after inoculation. Monkey 3 then received 0.25 g of sulfanilamide intraperitoneally on that day and 0.5 g on the following day. No drug was administered to Monkey 4, the control. Monkey 3 had a low-grade infection which subsided into a chronic form, while the control, Monkey 4, died of an overwhelming infection on the 9th day.

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[1] De Leon, A. D., Translation of original, *U. S. Pub. Health Rep.*, 1937, **52**, 1460.

[2] Hill, R. A., and Goodwin, M. H., *South. Med. J.*, 1937, **30**, 953.

[3] Read, H., and Pino, J. O., *Arch. Schiffs. u. Tropenhyg.*, 1938, **42**, 132.

[4] Van der Wielen, Y., *Nederl. Tijdschr. v. Geneesk.*, 1937, **81**, 2905.

* Supplied by the Winthrop Chemical Company, New York.

† Made by the Calco Chemical Company and obtained through the courtesy of Dr. A. R. Dochez.

Experiment 3. The foregoing experiments were repeated on 5 monkeys, Nos. 5 to 9, inclusive. These monkeys were each inoculated intraperitoneally with 20 million parasites, a larger number than previously employed, and at various intervals after inoculation they were given sulfanilamide. The procedure and results are summarized in Table I. It will be seen that Monkey 5, which received 0.5 g of sulfanilamide at the time of inoculation and on the 3rd and 5th days afterwards, never developed an infection. Monkey 6 received 0.5 g on the 3rd day, which was the first day that parasites appeared in the blood stream. Forty-eight hours later the parasites had disappeared. Monkey 7 was given 0.5 g on the 3rd day when the infection was well established and again on the 5th day. The course of the infection was severe but subsided rapidly without further therapy. Monkey 8 was treated with 0.5 g of sulfanilamide on the 4th day upon showing evidence of a rather intense infection with a count of 182 parasites per 10,000 red blood cells. This dose was repeated on the following day because the parasite count was increasing rapidly. After the second dose there was a marked improvement in the animal, and 48 hours later no parasites could be found in the blood smears. Control Monkey 9 died of acute malaria on the 10th day following inoculation. It was noted in the stained blood smears of those monkeys which had rapid declines in the number of parasites following sulfanilamide therapy that many of the parasites were distorted and broken. This observation indicated that the drug had a direct action on the parasites. The results of this experiment show that sulfanilamide in the amounts given was effective in preventing infection as well as in having a marked therapeutic value after the infection was established.

Experiment 4. In the following experiment the prophylactic effects of sulfanilamide were tested in 4 monkeys. Monkeys 10 and 11 each received intraperitoneally 0.5 g of the drug in a 10%

TABLE I.

Effect of Sulfanilamide Given Intraperitoneally at Various Intervals After Inoculation of Parasites.

Monkey	Sulfanilamide injections	No. of parasitized cells per 10,000 red blood cells. Days after inoculation.												
		1	2	3	4	5	6	7	8	9	10	11	12	13
5	.5 g on 1st, 3rd, and 5th days	0	0	0	0	0	0	0	0	0	0	0	0	0
6	.5 ’’ ’’ 3rd day	0	0	+	+	0	0	0	0	0	0	0	0	0
7	.5 ’’ ’’ 3rd and 5th days	0	+	12	143	1037	1188	210	0	0	0	0	0	0
8	.5 ’’ ’’ 4th and 5th days	0	+	8	182	990	25	0	0	0	0	0	0	0
9	Control, no drug given	0	+	16	129	480	980	1464	1500	2516	Dead			

TABLE II.

Prophylactic Effect of Sulfanilamide Given Intraperitoneally and by Mouth.

Monkey	Sulfanilamide injections	No. of parasitized cells per 10,000 red blood cells. Days after inoculation.											
		2	4	6	8	10	12	14	16	18	20	22	24
10	0.5 g in 4 daily doses*	0	0	0	0	0	0	0	0	0	0	0	0
11	0.5 ’’ ’’ 4 ’’ ’’ *	0	0	0	0	+	18	372	1575	1280	Dead		
12	2.0 ’’ by mouth†	0	0	0	0	0	0	0	0	0	0	0	0
13	2.0 ’’ ’’ ’’ †	0	0	0	0	0	0	0	0	10	32	780	86
14	Control, no drug given	0	3	840	1261	Dead							

*Final dose administered 24 hours before inoculation.

†Administered 24 hours before inoculation.

solution on 4 successive days before inoculation with malaria parasites, while Monkeys 12 and 13 each received 2.0 g by mouth in one dose. Twenty-four hours after the last administration of the drug these 4 monkeys and also Monkey 14, which received no drug and served as control, were each inoculated intraperitoneally with 20 million parasites. This represented approximately 10 million minimal infective doses.

The results of this experiment are shown in Table II, and it will be seen that of the 2 monkeys, Nos. 10 and 11, which received sulfanilamide intraperitoneally before inoculation of parasites, Monkey 10 failed to develop an infection. Monkey 11 became infected but did not show circulating parasites until 10 days following inoculation. It appeared to be recovering but finally succumbed on the 20th day with a marked anemia. Almost identical results were obtained in the 2 monkeys, Nos. 12 and 13, in which a single 2.0 g dose of sulfanilamide was given by mouth. Monkey 12 failed to become infected while Monkey 13 first had demonstrable parasites on the 17th day but recovered. Control Monkey 14 died of an overwhelming infection 9 days after inoculation of parasites. The outcome of this experiment indicates that sulfanilamide in the amounts given was responsible for preventing infection in Monkeys 10 and 12 and appreciably prolonging it in Monkeys 11 and 13.

Experiment 5. The *in vitro* action of sulfanilamide on the parasites was tested in 2 monkeys. Twenty million parasites were incubated with 0.1 g of the drug for 30 minutes at 37°C. This mixture was then injected into Monkey 15. Monkey 16 received a similar mixture except that the parasites were incubated with 0.05 g of sulfanilamide. Both monkeys failed to develop infections, indicating that the parasites had been completely inactivated *in vitro* by the drug.

Experiment 6. The therapeutic and prophylactic effect and the *in vitro* action of sulfanilyl sulfanilate were next studied. The method of administration and the results are summarized in Table III. It is seen from this table that sulfanilyl sulfanilate has some therapeutic value when given early in the course of an acute infection. No prophylactic effect is apparent, however, and *in vitro* the drug apparently has no deleterious effect upon the parasites comparable to that of sulfanilamide.

Experiment 7. Sulfanilamide and sulfanilyl sulfanilate were given to 4 monkeys with chronic infections in an attempt to effect a cure. Each drug was used separately in 2 monkeys which had previously been protected from death with immune serum and the duration of

TABLE III.

Prophylactic and Therapeutic Effect of Sulfanilyl Sulfanilate Given Intraperitoneally and Its Action upon Parasites *in vitro.*

Monkey	Sulfanilyl sulfanilate injections	No. of parasitized cells per 10,000 red blood cells. Days after inoculation.													
		2	4	6	8	9	10	11	12	14	16	18	20	22	24
17	0.5 g 1st to 5th days, incl.; 1.5 g on 9th day	0	0	0	0	12	180	102	82	210	300	80	40	20	12
18	0.5 g on 4th and 5th days	0	1	30	0	0	0	0	0	32	223	282	600	220	102
19	0.5 g on 1st, 3rd and 6th days	0	0	22	48	52	86	128	144	36	14	60	22	12	32
20	Control, no drug given	+	129	220	980	1440	1500	2536	Dead						
21	0.5 g in 4 daily doses*	0	+	150	855	1895	3596	Dead							
22	0.5 ” ” 4 ” ” *	0	+	812	1872	4560	Dead								
23	0.1 g incubated with parasites 30 min. at 37°C	0	0	2	32	324	2334	Dead							
24	Control, no drug given	0	3	840	1261	2582	Dead								

*Final dose administered 24 hours before inoculation.

their infection varied from 22 to 135 days. Both drugs were given in 0.5 g amounts for 6 successive days. Forty-eight hours after the last dose the animals were bled and 3 cc of blood from each monkey was subinoculated intravenously into separate normal monkeys. The blood from the 2 monkeys which received sulfanilamide was not infectious for normal monkeys while the blood from the sulfanilyl sulfanilate treated monkeys produced an infection in the normal animals.

Treatment of avian malaria. Sulfanilamide and sulfanilyl sulfanilate had no prophylactic effect or therapeutic value in the treatment of *P. cathemerium* infections in canaries or *P. lophuræ* infections in young chicks.

Summary. Sulfanilamide was found to have a prophylactic value and a marked therapeutic effect on acute *P. knowlesi* infections in rhesus monkeys. It had, in addition, a direct action upon the parasites *in vitro*.

Sulfanilyl sulfanilate proved an ineffective prophylactic agent; it had no apparent *in vitro* action on the parasites, but some therapeutic value in acute *P. knowlesi* infection was noted.

In chronic infections 3.0 g of sulfanilamide given intraperitoneally was sufficient to render blood non-infectious for susceptible monkeys, while the same amount of sulfanilyl sulfanilate was without effect.

Sulfanilamide and sulfanilyl sulfanilate were ineffective for prophylaxis and had no therapeutic value when tested against infections produced by 2 different species of avian plasmodia.

10011

Effect of Splenectomy on Response to Pituitary Material and the Question of the Antihormone.

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Although there is unanimity among investigators in the field that inhibitory substances appear in the blood following continuous treatment with various endocrine extracts, controversy still exists regarding the nature of these antihormone principles, and the mechanism responsible for their production. This controversy, in general, is confined to two schools of thought. The first adheres to the idea that antihormones are normally present in the blood of animals in varying amounts, the concentration of which is increased by hormone injection. Collip,[1] for example, compares the hormone-antihormone complex to a buffered system. (For a review of the more pertinent references on this subject, see Gordon.[2]) The other school believes the inhibitory substances are antibodies, evoked because of the association of the injected hormone with foreign protein.

One of the strongest arguments in favor of the antibody-like nature of these antagonistic substances has been the failure of animals receiving treatment with pituitary hormones of the same species to become refractory or develop antihormone substances. This has been demonstrated in different ways by numerous investigators.[3–10] Only Collip and his collaborators[11, 12] have reported refractoriness or inhibitory substances in rats and sheep treated with pituitaries of homozoic animals.

Recently, we[2, 13, 14] have shown that the so-called reticulo-endothelial system, known to be concerned with the production of antibody substances,[15] is intimately related to the state of refractoriness which develops after prolonged treatment with heterozoic endocrine extracts. A short time ago, Emery[16] reported experiments which demonstrated that splenectomy does not alter the ovarian response in rats receiving implants of rat pituitaries. Although this is the result one would expect if the antihormone substances are antibody-like, because of the great variation of ovarian weights in Emery's control and experimental groups of animals, we decided to repeat his experiment, using a method which would yield less variable results. At the same time, in order to settle the matter completely, we thought it advisable to conduct another series of experiments to determine whether injections of pituitary material, prepared in the same way, but from a heterozoic source, would produce any different effect in normal as compared to splenectomized animals.

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[9] Katzman, P. A., Wade, N. J., and Doisy, E. A., *Endocrinol.*, 1937, **21**, 1; PROC. SOC. EXP. BIOL. AND MED., 1938, **38**, 122.

[10] Thompson, K. W., PROC. SOC. EXP. BIOL. AND MED., 1937, **35**, 634.

[11] Selye, H., Collip, J. B., and Thomson, D. L., PROC. SOC. EXP. BIOL. AND MED., 1934, **31**, 566.

[12] Collip, J. B., *Can. Med. Assn. J.*, 1937, **36**, 199.

[13] Gordon, A. S., Kleinberg, W., and Charipper, H. A., *Science*, 1937, **86**, 62; PROC. SOC. EXP. BIOL. AND MED., 1937, **36**, 484; *Anat. Rec.*, Supplem. No. 1, 1937, **70**, 49.

[14] Gordon, A. S., and Charipper, H. A., *Anat. Rec.*, Supplem. No. 3, 1938, **70**, 31.

[15] Perla, D., and Marmorston, J., *The Spleen and Resistance*, Williams and Wilkins Co., 1935.

[16] Emery, F. E., PROC. SOC. EXP. BIOL. AND MED., 1937, **37**, 455.

Rat pituitaries were obtained from 135 male animals (150-200 g) castrated approximately 2 months before the experiments were begun. Castration was performed to increase the gonadotropic potency of the glands. On the days of injection, 15 of these animals were killed, the pituitaries removed, and minced with fine scissors to yield a pulp which could be taken up in a small amount of saline for injection purposes. Nineteen control and 19 splenectomized immature (25-30 days old) female rats from the same litters were injected intraperitoneally with 5 mg of this whole rat pituitary material 9 times, the injections being evenly distributed over a period of 25 days.

Sheep pituitaries from freshly slaughtered animals were employed for the second part of the experiment. As with the rat pituitaries, this material was minced up finely and then mixed with a small amount of saline for injection. Ninety mg of whole sheep pituitary prepared in this way, when administered 9 times, was found to have approximately the same effect on the ovarian weight of the immature rat as nine 5 mg injections of the whole castrate rat glands. Twenty normal and 17 splenectomized immature female rats from the same litters were given nine 90 mg intraperitoneal injections, distributed over a period of 25 days. All rats employed in this study were from a highly inbred strain and were free of *Bartonella muris*.

Twenty-six days after beginning treatment all the animals were sacrificed and the ovarian weights determined. Similarly the uteri were cleanly dissected, pressed on a piece of blotting paper to expel any fluid, and the weights measured. The ovarian and uterine weights obtained in these experiments together with a statistical analysis of the results are given in Table I. The ovarian and uterine weights in 12 normal untreated 52-55-day rats are also included.

From an observation of this table, the following conclusions may be reached: (1) Removal of the spleen does not alter the ovarian and uterine weight response to pituitary material from homozoic

TABLE I.

Ovarian weights (mg).

Animal	Pituitary inj.	No. of animals	No. of inject.	Mean ovarian wt. ± S.E.	Range	Mean diff. / S.E. of diff.	Coeff. of variation
Normal	Castrated ♂ rat	19	9	40 ± 2.2	22-58	1.3	23.7
Splenect.	,, ♂ ,,	19	9	36 ± 2.1	24-56		24.7
Normal	Sheep	20	9	34 ± 2.2	20-48	6.4	29.1
Splenect.	,,	17	9	52 ± 1.8	39-65		14.2

Uterine weights (mg).

Animal	Pituitary inj.	No. of animals	No. of inject.	Mean uterine wt. ± S.E.	Range	Mean diff. / S.E. of diff.	Coeff. of variation
Normal	Castrated ♂ rat	19	9	112 ± 6.3	66-161	0.89	24.2
Splenect.	,, ♂ ,,	19	9	105 ± 4.8	72-145		19.4
Normal	Sheep	20	9	85 ± 4.6	52-125	6.7	24.2
Splenect.	,,	17	9	142 ± 7.2	105-197		20.8

Ovarian and uterine weights (mg) in normal 52-55-day rats.

No. of animals	Mean ovarian wt. ± A.D.	Mean uterine wt. ± A.D.
12	16 ± 2.3	52 ± 10.6

S.E.—Standard Error. A.D.—Avg Deviation.

animals. This may be seen from the values of the standard errors, and the fact that the value of the difference between the means divided by the standard error of the difference between the means is less than 2. This would be in agreement with the conclusions reached by Emery. (2) The statistical analysis, however, reveals that splenectomized rats, injected with pituitary from heterozoic animals, show a greater increase in ovarian and uterine weights than normal rats similarly treated.

These results are in total accord with the general contention that the refractory state which develops after prolonged treatment with heterozoic endocrine material is brought about by a mechanism similar to that responsible for the production of antibodies following injection of antigenic material.

It may be argued that the effects obtained with the sheep pituitary material are due to the greater quantities of sheep as compared to the amounts of castrate rat pituitary injected. As has already been pointed out, however, the sheep and rat material produced approximately the same intensity of effect on the ovarian and uterine weights. That the amount of protein injected does not determine the difference may be seen from some of our earlier experiments[2] which show that great differences in effect in splenectomized and control animals are obtained with the use of small amounts of an endocrine preparation (Follutein) which contains very little protein.

Summary. Splenectomy in rats is without effect on the ovarian and uterine weight response to injections of homozoic pituitary material. Splenectomized rats, however, show greater response when treated with pituitary material from a heterozoic source than similarly treated controls. This lends further support to the contention that the state of refractoriness which develops after prolonged treatment with hormone preparations is immunological in nature.

10012

Effect of Parathyroid Extract upon Volume of Parathyroid Glands in Normal and Partially Nephrectomized Rats.

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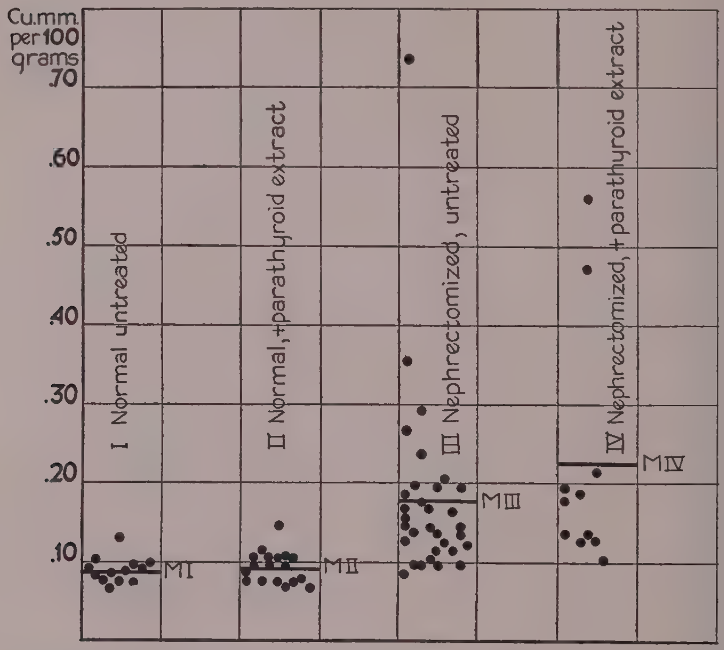
In the course of certain experiments upon sensitiveness of partially nephrectomized rats to parathyroid extract, an opportunity



Parathyroid Volume
in cu. mm.

was offered to ascertain whether the administration of this hormone over shorter or longer periods leads to a reduction in the size of the parathyroid glands. It is a commonly held belief that substitution therapy brings about functional atrophy of the endocrine glands producing the secretion. In the case of the parathyroids, Jaffé and Bodansky[1] have reported that in dogs, the size of the parathyroid was reduced from one-third to one-half of that usually found; and that there were microscopic evidences of atrophy after acute or chronic experimental hyperparathyroidism. However, they did not weigh or measure the glands, and it seemed desirable to obtain more precise information on the subject.

White and hooded rats were maintained on a standard diet.[2] Parathyroid extract (Lilly) was given by intraperitoneal injection in total doses ranging from 200 to 2150 Hansen units over periods of from 3 to 51 days. The combined volume of the parathyroid glands, after short fixation in Zenker, was measured from serial sections.[3] Determinations were made on 4 groups of rats:

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[1] Jaffé, H., and Bodansky, A., *J. Exp. Med.*, 1930, **52**, 669.

[2] Donohue, W., Spingarn, C., and Pappenheimer, A. M., *J. Exp. Med.*, 1937, **66**, 697.

Group (I) Normal, untreated controls.
" (II) Normals, treated with parathyroid extract.
" (III) Partially nephrectomized, untreated rats.
" (IV) Partially nephrectomized rats, injected with parathyroid extract.

The individual data are shown on the scatter chart, and the mean values in the various groups are presented in Table I.

TABLE I.

Group	No. of rats	Mean volume of parathyroids in mm³ Abs.	Per 100 g
I	13	.1483	.0886
II	18	.1506	.0914
III	32	.2314	.1789
IV	11	.2413	.2222

It is obvious that the injection of parathyroid extract, even in large doses, does not bring about an involution of the normal glands, nor does it prevent the hyperplasia which follows partial nephrectomy.

We are greatly indebted to the Eli Lilly Co. for the parathyroid extract used in these experiments.

10013

Effect of Testosterone Propionate upon Endometrial Cycle of the Human.

J. A. Gaines, U. J. Salmon and S. H. Geist.

From the Gynecological Service of Dr. S. H. Geist and Department of Laboratories of the Mt. Sinai Hospital, New York City.

Experimental evidence is accumulating which indicates that male hormone counteracts the physiologic effects of estrogenic hormone. Ihrke and D'Amour[1] reported suppression of the estrous cycle in female rats while being injected with male hormone concentrates prepared from bull testes. Similar results were obtained by Robson[2] in mice and Browman[3] in rats with testosterone. Zuckerman[4] has reported that testosterone propionate inhibits follicle growth and luteinization in monkeys. The present investigation was undertaken to determine the effect of testosterone propionate upon the endometrial cycle of the menstruating human female.

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[3] Jarrett, W. A., Peters, H. A., and Pappenheimer, A. M., Proc. Soc. Exp. Biol. and Med., 1935, **32**, 1211.

[1] Ihrke, I. A., and D'Amour, F. E., *Am. J. Physiol.*, 1931, **96**, 289.

[2] Robson, J. M., Proc. Soc. Exp. Biol. and Med., 1936, **35**, 49.

A group of 6 women were selected and preliminary control endometrial (suction) biopsies were performed in each case to determine the presence of a normal endometrial cycle. In all of the cases selected for this study, the control biopsies performed during the latter half of the cycle showed, on one or more occasions, an endometrium in the secretory phase, indicating a regular estrogenic and corpus luteum effect.

These women were given testosterone propionate* in individual doses of 25 to 100 mg at intervals of 2 to 3 days. The total monthly dosage was varied from 175 mg to 800 mg. The androgen was administered intramuscularly in concentrations of 25 to 50 mg per cc of sesame oil. During the course of testosterone injections, suction biopsies of the endometrium were performed at intervals of 1 to 4 weeks, covering 1 to 3 monthly cycles.

Because of the lack of uniformity in the terminology employed in the literature to describe the morphologic variations of the endometrium, a brief description of the histologic criteria employed in this study is presented.

The normal monthly cycle may be divided into menstrual, proliferative and secretory phases. The *proliferative* phase occurs from the end of menstruation to the time of ovulation. In a 28-day cycle, this extends over a period of approximately 10 days, following a 4-day menstrual period. The endometrium is characterized by an increase in thickness, enlargement and tortuosity of the glands, numerous mitoses, pseudostratification of the epithelial cells, and increase in the number, size and mitotic activity of the stroma cells. There is abundant experimental evidence to indicate that this phase is the result of purely estrogenic stimulation of the endometrium resulting in progressive cellular activity and growth.

The *secretory* phase occurs from the time of ovulation to the beginning of menstruation. This phase is characterized by loss of epithelial pseudostratification, early realignment of the glandular epithelium with centralization of the nuclei, presence of subnuclear vacuolization and glycogen accumulation, papillary projections of the glandular epithelium into the gland lumen, corkscrew outline of the glands, rare mitoses, and secretion within the gland lumen. The stroma shows increasing edema and lymphocytic infiltration. The stroma cells are enlarged and many of them contain glycogen. The glycogen stain, when used, is of great value in differential diagnosis, since the proliferative phase does not show the presence of glycogen except in the slightest degree. These secretory endometrial changes are the result of a combined estrogen and progesterone effect.

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[3] Browman, L. G., PROC. SOC. EXP. BIOL. AND MED., 1937, **36**, 205.

[4] Zuckerman, S., *Lancet*, 1937, **2**, 676.

* We are indebted to Drs. Gregory Stragnell and Erwin Schwenk of the Schering Corporation for the testosterone propionate.

The term *hyperplastic* endometrium indicates an abnormally marked estrogenic effect. It excludes the presence of any corpus luteum stimulation. It represents a hyperproliferation beyond that seen in the average, late proliferative phase of a normal cycle.

The endometrial changes noted after the administration of testosterone propionate are the reverse of those observed in hyperplasia. In contrast, they suggest an inhibition or suppression of estrogen stimulation and proliferation. The term *hypoplasia* is proposed to describe a subnormal proliferative effect, that is, less than that seen in the average, early proliferative phase of the normal cycle. When the manifestations of cellular activity and growth are more strikingly reduced, the designation *marked hypoplasia* is used. *Atrophy*

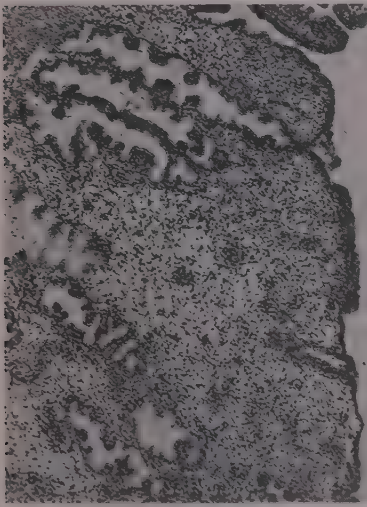

Fig. 1.

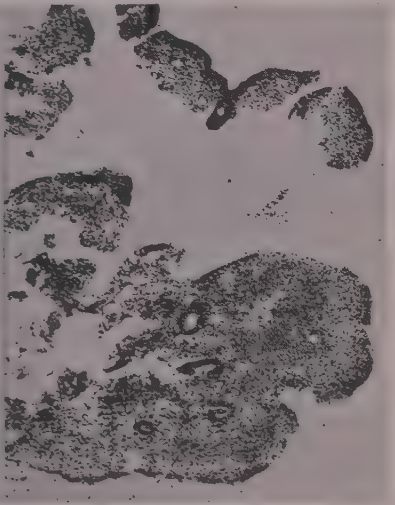

Fig. 2.

Fig. 1.

Control suction curettage—taken premenstrually. Endometrium showing normal secretory phase.

Fig. 2.

Atrophic endometrium after the administration of testosterone propionate.

is suggested for the endometrial picture resembling that encountered long after the menopause of castration (Fig. 1).

With atrophy, only minute fragments of tissue are obtained despite vigorous curettage with the suction curette. The endometrium is reduced to a thin strip, as evidenced by the not infreqent presence of myometrial tissue a short distance below the surface epithelium. Fragmentation of the specimen is common in contrast to the well-preserved strips removed during normal phases. The endometrial glands are few in number and narrow or slightly dilated. The glandular epithelium does not show the presence of mitoses or pseudostratification. The epithelial nuclei are small, irregular in contour, dark staining, and often pyknotic. Vacuolization of the cytoplasm and other evidences of plasmolysis are present. The stroma cells are not abundant and are spindle shaped, with small, dark nuclei. No mitoses are to be seen within the stroma cells.

In hypoplasia, the endometrium shows signs of definitely reduced cellular activity. The mitotic figures ordinarily seen within the stroma cells and glandular epithelium of the early proliferative phase are rare. The epithelial nuclei are not uniform, light staining or vesicular, but small, dark and often irregular. Evidence of cellular degeneration is frequent. The cytoplasm is more acidophilic. The stroma cells are small and dark staining. The picture is that of a quiescent or partly regressive state. Depending upon the extent to which the endometrium approaches the picture of atrophy, the terms slight or marked hypoplasia are used.

After the administration of testosterone propionate, the endometrial biopsies in all the 6 cases revealed absence of secretory phase or progesterone effect. In 2 of these cases the endometrium presented a picture of atrophy; in one, marked hypoplasia; in 2, slight hypoplasia; and in another, slight proliferation. Pseudomenstruation (bleeding from a non-secretory phase) occurred after the first month of testosterone injections in 2 cases. In a third case, very slight bleeding occurred on 3 occasions at regular monthly intervals. With continued testosterone administration, all of the 6 patients subsequently missed one or 2 menstrual periods.

It appears from these studies that after the administration of adequate doses of testosterone propionate, the following striking morphologic changes occur in the endometrium of cyclical women: (1) Disappearance of the secretory phase; (2) inhibition of the proliferative phenomena, often with regression to the hypoplastic or atrophic state.

These results may be interpreted as indicating either inactivation of estrogen and progesterone or inhibition of follicle and corpus luteum development. Salmon[5] has shown that the hyperactive hypophysis of the human female castrate can be inhibited with testosterone propionate. It seems logical to assume, therefore, that the suppression of estrogen and progesterone effects in the endometrium, which occurred in the cases reported here, following the administration of testosterone propionate, may be brought about by a similar mechanism—*viz.*, inhibition of the gonadotropic factors (both follicle stimulating and luteinizing) of the hypophysis.

Loeser[6] reported the observation that diminution in bleeding occurred in cases of metrorrhagia following administration of testosterone propionate. He cites a case of irregular menstruation associated with endometrial hyperplasia in which the endometrium became atrophic after 500 mg of testosterone propionate, and suggests inhibition of the hypophysis as a possible explanation of the action of the testosterone propionate.

10014

Effect of Progesterone on Fallopian Tube Contractility.

S. H. GEIST, U. J. SALMON AND M. E. MINTZ.

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It has been previously shown that the Fallopian tubes in women after the menopause lose the rhythmic contractions which, in cyclical women, can be recorded on a kymograph.[1] It was further shown that regular rhythmic contractions can be restored to the Fallopian tubes by the administration of adequate doses of estrogenic hormone.

In the present investigation, an attempt was made to evaluate the effect of progesterone on the Fallopian tube contractility. Five postmenopause women were selected whose vaginal smear studies revealed definite estrogen deficiency. Preliminary control kymographic recordings of the tubal contractions were obtained at weekly intervals, employing the Rubin insufflation apparatus.[2] The patients were then each given a course of estrogen injections in the form of estra-

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[5] Salmon, U. J., PROC. SOC. EXP. BIOL. AND MED., 1937, **37**, 488.

[6] Loeser, A. A., *Lancet*, 1938 (Feb. 12), 373.

[1] Geist, S. H., Salmon, U. J., and Mintz, M. E., *Am. J. Obst. and Gyn.*, in press.

[2] Rubin, I. C., *J. A. M. A.*, 1920, **75**, 661; 1928, **90**, 99.

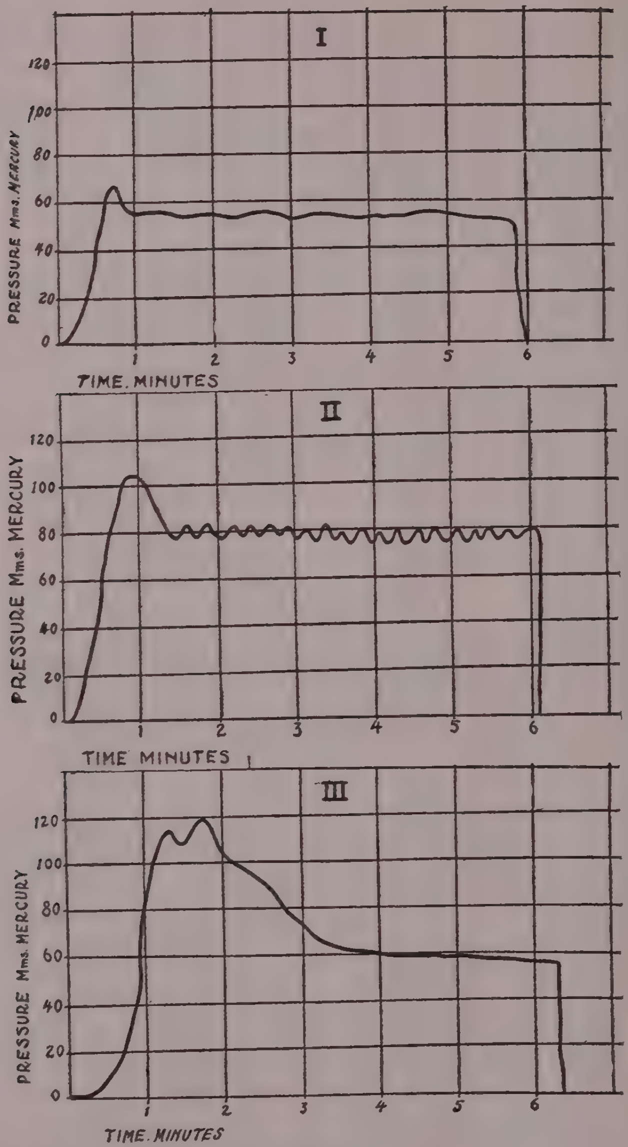

FIG. 1.

Case M.K. Age 47. Last menstrual period 18 months before.

I. Preliminary recording, showing absence of tubal contractions.

II. Tubal contractions after 820,000 I.U. estradiol-benzoate.

III. Recording taken 2 hours after tracing (II) and after 20 mg progesterone—revealing inhibition of tubal contractions.

diol-benzoate* (Progynon-B). The total dosage varied from 450,000 to 900,000 I.U. given intramuscularly in divided doses over a period of 10 days. At the end of the course of estrogen injections, recordings of tubal contractions were again obtained. In each case the preliminary control recording revealed a complete absence of tubal contractions and, after the estrogen administration, regular rhythmic fluctuations appeared.

Immediately following the last recording of tubal contractions, the patients were given 20 mg of progesterone* (Proluton) intramuscularly in 4 cc of sesame oil. The patients were then again insufflated 2 hours later. The recording taken 2 hours after the administration of the progesterone revealed a complete absence of pressure fluctuations, indicating inhibition of tubal contractions. The kymographic recordings of a typical case are presented in Fig. 1.

It would appear from the results of these experiments that the Fallopian tube contractions which are induced by estrogenic hormone can be inhibited by progesterone.

10015

Assay of a Vitamin K Preparation for Vitamin D.

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Vitamin K preparations have been used as therapeutic measures in the treatment of the bleeding tendency in patients with jaundice.[1, 2, 3] McNealy, Shapiro, and Melnick[4] demonstrated that the administration of Vitamin D (Viosterol) to patients with jaundice leads to a marked decrease in the "venostasis bleeding time."[5] This work has been confirmed by Boys.[6] Both these vitamins are fat soluble, and bile or bile salts are administered with them, when given to jaundiced patients, because bile salts appear to be essential for the absorption of both.[1-3, 7, 8] Regardless of the method of preparation of the Vitamin K being used clinically, we thought it advisable to assay the preparation for Vitamin D content to ascertain if the clinical results might be attributed to Vitamin D. Accordingly, a sample of a Vitamin K preparation prepared by Dr. Dann of the Abbott Laboratories was obtained. This preparation of Vitamin K would protect chicks from developing avitaminosis K when fed at a level of 0.01% of the diet. This preparation was then subjected to the U. S. P. XI biological test for Vitamin D.

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* For the Progynon-B and Proluton used in these experiments, we are indebted to Drs. Gregory Stragnell and Erwin Schwenk of the Schering Corporation, Bloomfield, N. J.

[1] Warner, E. D., Brinkhous, K. M., and Smith, H. P., PROC. SOC. EXP. BIOL. AND MED., 1937, **37**, 628.

[2] Snell, A. M., *Proc. Staff Meetings Mayo Clinic,* 1938, **13**, 65.

[3] Butt, H. R., Snell, A. M., Osterberg, A. E., *Proc. Staff Meetings Mayo Clinic,* 1938, **13**, 74.

[4] McNealy, R. W., Shapiro, P. F., and Melnick, P., *Surg. Gynec. and Obst.,* 1935, **60**, 785.

[5] Ivy, A. C., Shapiro, P. F., and Melnick, P., *Surg. Gynec. and Obst.,* 1935, **60**, 781.

Twenty-four rats, 28 days of age, were divided into 3 groups and placed in individual cages on a rachitogenic diet. One animal from each group was sacrificed at the end of the depletion period (25 days) to demonstrate the degree of the rickets—4+ in each group.

Group 1 received 5 mg (1/40th of the human dose) of the Vitamin K concentrate in 0.1 cc of peanut oil daily for 8 days. Group 2 received 0.5 international units of Vitamin D as Viosterol for 4 days, then 1 unit daily for the succeeding 4 days. This was administered in 0.1 cc peanut oil daily also. Group 3 received 0.1 cc oil daily for 8 days. All groups were removed from medication on the ninth and tenth days, and were killed on the eleventh day following the beginning of treatment.

Group 1 (Vitamin K treated) showed no healing at the end of the period. Group 2 (Vitamin D treated) showed 3 rats with 3+ healing and 4 rats with 4+ healing at the end of the period. Group 3 (peanut oil treated) showed no improvement at the end of the period; all were rachitic.

Summary. It is evident, then, that, in the doses given, this preparation of Vitamin K known to be potent in the treatment of the hemorrhagic diathesis of chicks does not contain enough Vitamin D to cause healing in rachitic rats.

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[6] Boys, F., *Surgery*, 1937, **2**, 817.

[7] Greaves, J. D., and Schmidt, C. L. A., *Am. J. Physiol.*, 1935, **111**, 492.

[8] Greaves, J. D., and Schmidt, C. L. A., *Univ. Calif. Pub. Physiol.*, 1934, **8**, 43.

10016

Effect of Liver Extract, Injected Parenterally into Pregnant Mother, on Erythrocytes of Newborn Rats.

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From the Department of Physiology and Pharmacology, Northwestern University Medical School, Chicago.

A report by Stasney, Higgins, and Mann[1] indicated that the parenteral administration of the concentrated gastric juice of man and swine into the pregnant albino rat caused a reduction of the maximal size of the erythrocytes of the newborn pups. The results suggested that the reduction in the size of the erythrocytes was due to the presence of an anti-pernicious anemia factor. If that were true, we thought that the effect might serve as a laboratory test for the presence of the anti-pernicious anemia factor in liver extract. Accordingly, an experiment has been performed to test such an hypothesis, in which we have directed our attention to the mean, rather than the maximal, diameter or size of the erythrocytes.

Our stock albino rats were used, which are an inbred strain derived from a cross between a Wistar and Chicago alley rat. The pregnant rats were placed in individual cages and fed a diet of Purina dog biscuits and powdered milk, water being available at all times. Ninety newborn of 12 pregnant rats served as controls. Seventeen other pregnant rats were injected intraperitoneally with 0.2 cc of a liver concentrate, a relatively large dose, from 2 to 14 times on separate days prior to delivery. These rats delivered a total of 110 pups. The liver concentrates used were obtained from Eli Lilly and Company (Reticulogen) and from Chappel Brothers (Liver Extract Concentrated), and was known to be potent by actual tests on patients having pernicious anemia. No ill effects from the injections were observed. The blood of the pups of the treated and untreated mothers was collected and studied usually within 2 hours after birth. The blood was obtained by cutting off the tail. The erythrocytes were enumerated with a standardized pipette. Because of the small amount of blood, hematocrit determinations were made on the combined heparinized blood of 3 pups by the method of Rosahn.[2] Hemoglobin was estimated by the New-

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[1] Stasney, J., Higgins, G. M., and Mann, F. C., *Proc. Staff Meetings Mayo Clinic*, 1937, **12**, 699.

[2] Rosahn, P. D., Proc. Soc. Exp. Biol. and Med., 1931, **28**, 491.

TABLE I.

No. of Injections		No. of litters	No. of pups	R.B.C. mil.	Hb. g per 100 cc.	Hematocrit	Mean Corp. Vol.	Mean red-cell diam.	A Max.* Mean Size	Min. Mean Size	B Avg† diam. largest cells	C Avg diam. smallest cells
Control	— 0	12	90	2.68	13.44	38.3	146	9.08 ± .06	11.72	8.23	11.22	7.82
Treated	— 2	2	13	2.74	14.28	44.8	163	9.14 ± .12	9.64	8.11	11.46	7.54
”	— 3	1	8	2.74	14.26	37.0	135	9.15 ± .10	9.61	8.69	11.29	7.84
”	— 4	1	8	2.72	12.90	39.3	144	9.26 ± .11	9.72	8.61	11.43	7.49
”	— 5	2	11	2.33	11.30	38.8	166	8.70 ± .08	9.15	8.29	10.57	7.50
”	—Avg	6	40	2.62	13.16	40.5	154	9.07 ± .10				
”	— 6	4	26	2.80	12.98	42.1	150	8.95 ± .05	9.24	8.37	11.16	7.85
”	— 7	1	8	2.97	12.84	37.0	124	9.04 ± .05	9.29	8.87	11.35	7.99
”	— 8	1	7	2.79	14.46	—		8.95 ± .11	9.35	8.47	10.66	7.95
”	— 9	1	3	2.71	14.58	40.0	147	8.81 ± .03	8.85	8.76	11.41	6.71
”	—11	1	6	2.71	14.04	48.0	177	9.13 ± .45	9.55	8.71	11.41	8.10
”	—12	1	4	2.38	11.48	—		9.12 ± .09	9.36	8.98	11.78	7.88
”	—13	1	9	2.94	12.84	—		8.84 ± .10	9.33	8.46	11.36	7.65
”	—14	1	7	2.50	12.62	42.4	169	9.02 ± .07	9.32	8.70	11.82	7.34
”	—Avg	11	70	2.77	13.13	41.9	151	8.97 ± .12				

*The maximum mean red-cell size observed in any newborn rat.

†The average diameter of the largest red cell measure in all newborn rats.

comer method. The mean red cell diameters (Price-Jones counts) were determined under oil immersion (1212X) by a standardized and calibrated disc on blood smears stained with Wright's stain, 100 consecutive cells were measured. Only very thin smears, or smears in which the red cells did not touch one another, were used.

The averaged results are shown in Table I. The normal mature rats in our colony have a mean erythrocyte count of approximately 9.0 million, 14.5 g of hemoglobin per 100 cc of blood, and a mean red cell diameter of 6.29 micra. When these figures are compared with those for the newborn rats, it is evident that the newborn rat has an erythopenia which is compensated for by a macrocytosis, since the hemoglobin values of the newborn are only slightly less than that of the adult. However, the blood picture of the newborn rats was not significantly altered by antenatal injection of the mother with liver extract of known potency. The mean red cell diameter of the pups of the untreated controls was 9.08±0.06 micra (range 11.72 to 8.23); in the pups of the mothers receiving up to 6 injections it was 9.07 micra; in the pups of the mothers receiving 6 to 14 injections it was 8.97±0.12 micra. It is to be noted that the wide range observed in the diameters of the erythrocytes (or the anisocytosis) of the untreated newborn was reduced by the treatment, although the mean diameter, mean corpuscular volume, and other values were not significantly changed. However, there were but 3 normal pups examined whose mean red cell diameters exceeded 9.74 micra (these were 11.72, 10.44, and 10.78 micra).

The mean red cell diameters observed by us for newborn rats (9.07 micra) is at variance with that reported by Stasney, *et al.*,[3] Wills,[4] and Smith.[5] Stasney, *et al.*, obtained a value of 9.54 micra, Wills a value of 8.62 micra on only 4 newborn rats, and Smith a value of 8.65 micra on only 5 newborn rats. Our mean determinations of the number of erythrocytes agree with those of Jolly (2.31),[6] Chisholm (2.69),[7] Jolly (2.76),[8] Sure, *et al.*, (2.8),[9] and Kindred and Corey (2.48).[10] However, the hemoglobin values vary to a greater extent. The differences observed may be due to a difference in diet, breed, or technic. Regardless of the differences in the mean red cell diameters of our newborn rats and those of Stasney, *et al.*, we doubt that there is a trend toward a decreased mean red cell size in the newborn rats of the mothers receiving injections of liver extracts known to contain the anti-pernicious anemia substance.

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[3] Stasney, J., and Higgins, G. M., *Proc. Staff Meetings Mayo Clinic*, 1937, **12**, 490.

[4] Wills, L., and Mehta, M. M., *Ind. J. Med. Res.*, 1930, **18**, 307.

[5] Smith, C., *J. Path. and Bact.*, 1932, **352**, 717.

[6] Jolly, J., *Comp. Rend. Soc. Biol.*, 1909, **2**, 136.

[7] Chisholm, R. A., *J. Exp. Physiol.*, 1911, **4**, 207.

[8] Jolly, J., *Comp. Rend. Soc. Biol.*, 1919, **82**, 800.

[9] Sure, B., Kik, M. C., and Walker, D. J., *J. Nutrition*, 1929, **1**, 299.

[10] Kindred, J. C., and Corey, E. L., *Anat. Rec.*, 1930, **47**, 213.

These results are compatible with the findings of Wigodsky, Richter, and Ivy,[11] that fetal livers of calves are abundantly filled with the anti-pernicious anemia factor. Hence, it can be hypothesized that the addition of more anti-pernicious anemia factor to hemopoietic systems already well supplied should incite no differences in the erythrocyte picture of the animals. These data bear out this hypothesis. Our results should not be interpreted as implying that gastric juice concentrates are ineffective or that our results and those of Stasney, *et al.*,[3] are contradictory.

Wintrobe, *et al.*,[12] have reported similar results in rabbits. They could observe no change in the red blood cell size of the newborn rabbits whose mothers had been injected intramuscularly with a potent liver extract. In one case they succeeded in injecting the extract into one uterine horn; the red blood cells of the fetuses of the injected horn did not differ significantly from those of the fetuses of the uninjected horn at term.

Summary. Data concerning the erythrocyte picture of newborn rats whose mothers had been treated antenatally with liver extracts known to contain the anti-pernicious anemia factor has been presented and shown to be not statistically different from that of untreated controls; or the antenatal injection of liver extract into a pregnant rat has no significant effect on the mean red cell diameter of the newborn rats.

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[11] Wigodsky, H. S., Richter, O., and Ivy, A. C., *Proc. Am. Phys. Soc.*, 1938, 214.

[12] Wintrobe, M. M., Kinsey, R. E., Blount, R. E., and Trager, W., *Am. J. Med. Sc.*, 1937, **193**, 449.

10017 P

On the Nature of the Toxic Action of Vitamin D.

C. I. Reed.

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Early experience with incompletely purified preparations of vitamin D demonstrated a high degree of toxicity which was first ascribed to by-products such as toxisterol and tachysterol. Later calciferol also was found to be toxic in large doses. While hypercalcemia commonly occurs with intoxication from calciferol, viosterol, toxisterol or tachysterol, it has been amply demonstrated that hypercalcemia is not *per se* the cause of toxicity.

The fact that the symptom complex of intoxication is identical, qualitatively, when produced by any of these agents leads to a tentative assumption that intoxication in each case may not be due fundamentally, to hypervitaminosis D, but to the formation of toxic products either in the alimentary canal or in the process of intrinsic metabolism when an excess of the vitamin in the form of calciferol or viosterol has been administered.

Crystalline ergosterol dissolved in ether was irradiated with a quartz mercury vapor lamp, according to directions kindly supplied by Dr. F. G. McDonald. One lot assayed by the line test yielded 9,000,000 (±15%) units per gram of original ergosterol. A part of this was recovered from the ether without purification; consequently the material comprised a mixture of calciferol, ergosterol and other products in unknown proportions.

A portion of this material was then further irradiated and on recovery without purification yielded, on assay, 3,000,000 (±15%) units of vitamin D per gram of original ergosterol, showing that there had been further decomposition so that there must have been still a larger proportion of other products and less unchanged ergosterol (Gp II).

Next, a solution of calciferol in oil, 1,000,000 units per gram of solution, was allowed to stand open in a warm room until it became cloudy and the quality of taste was markedly altered. On assay this was found to contain only 50,000 units per gram (Gp III). In Table I are shown the results of experiments on dogs with these preparations.

Group I comprised 12 male dogs of comparable age, weight and physical condition. These were given daily by mouth 12,000 vitamin D units of the first preparation per kilo of body weight. Of

TABLE I.

Group	No. dogs	Vitamin D I.U./kg/day	No. toxic in 15 days	Assay of preparation. Units per g of original ergosterol
I	12	12,000	7	9,000,000 (±15%)
II	14	4,000	11	3,000,000 (±15%)
III	13	800	9	[*]
IV	14	20,000	0	[†]

[*] Viosterol (1,000,000 units per gram, 16,000,000 units per gram of original ergosterol). Allowed to decompose until assayed at 50,000 units per gram.

[†] Viosterol (1,000,000 units per gram). Stock solution prepared from material practically free from toxisterol, but containing a small amount of unchanged ergosterol.

these, 7 showed definite evidences of intoxication in 15 days, when the experiment was discontinued.

Group II comprised 11 male and 3 female dogs, comparable to those in Group I. These were fed daily 4,000 units of vitamin D per kilo in the form of the second preparation, which had been overirradiated. Of these, 9 males and 2 females were intoxicated in 15 days.

Group III consisted of 8 males and 5 females. These were fed daily of the decomposed material an amount sufficient to make up 800 vitamin D units per kilo. Of these, 3 females and 6 males became toxic in 15 days.

Group IV. Of 14 dogs receiving daily 20,000 units of vitamin D per kilo in the form of viosterol (1,000,000 units per gram), none showed any symptoms of intoxication within 15 days, yet they received the largest dose in vitamin D units.

These experiments strongly suggest that the degradation products were responsible for the intoxication. It is possible that the intoxication resulting from doses of calciferol above 20,000 units per kilo per day may be due to formation of similar compounds when the organism is overloaded with the vitamin.

10018

Metabolism Body Size and Age in Baby Chicks.

MAX KLEIBER.

*From the College of Agriculture, University of California, Davis.**

The metabolic rate of homeotherms is determined by body size to such a degree that for a great variety of animals from rat to steer the daily heat production in kilocalories during fasting can be predicted by the same formula, namely, $72\ W^{3/4}$, in which W stands for the body weight in kilograms.[1]

Kleiber and Dougherty[2] have observed that the metabolic rate per $kg^{3/4}$ of abundantly fed baby chicks increased with increasing age. As a general rule the metabolism of the full fed animal parallels the fasting katabolism.[3] It was, therefore, assumed that the fasting katabolism per $kg^{3/4}$† of baby chicks increases also with increasing age which would mean a deviation from either ¾ power—or surface law of metabolism. This assumption has been confirmed by 2 series of respiration trials. In one series (30° series) 5 groups of 10 chicks per group were used, representing the ages of 12, 14, 16, 18, and 20 days. The metabolic rate was measured during 12 hours from 8 p.m. to 8 a.m., starting 24 hours after withdrawal of food. The temperature during fast and respiration trial was kept at 30°C, the humidity at approximately 50%. The apparatus used was the same as that described earlier.[2]

In a second series (35° series) 6 groups of 18 chicks represented the ages of 1, 5, 8, 12, 16, and 20 days. The metabolic rate was measured during 4 hours, 11 a.m. to 3 p.m., starting 17 hours after withdrawal of food. For the duration of the fast and respiration trial the temperature was kept at 35°C. During the respiration trial the humidity was close to saturation. (In earlier unpublished trials we had found that very wide variations in humidity did not measurably affect the metabolic rate of baby chicks at temperatures below 37°C.) The apparatus used for the measurement of the respiratory exchange has been constructed at our station for serial work with rats. It consists of 7 respiration chambers, each connected to a rocking absorbing battery and a burette filled with oxygen. Water from a Mariotte bottle runs into these burettes and drives oxygen to the chambers at the same rate as it is consumed by the animal. Three chicks were put into each of 6 chambers, one chamber was used as a blank.

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* The author acknowledges the help of Dr. T. Jukes, Poultry Division, University of California.

[1] Kleiber, M., *Hilgardia*, 1932, **6**, 315.

[2] Kleiber, M., and Dougherty, J. E., *J. Gen. Physiol.*, 1934, **17**, 714.

[3] Kleiber, M., *Tierernährung*, 1933, **5**, 6.

† $kg^{3/4}$ means the unit of body weight in kilograms raised to the ¾ power.

In 9 trials R.Q.'s higher than 0.75 were found. We omitted the data of these trials from our calculations. However, we found that the final result would have been essentially the same if it had included these data.

The daily heat production per $kg^{3/4}$ is plotted against age in Fig. 1, together with results calculated from data published by Barott and his coworkers.[4]

Fig. 1 shows that the influence of age on the metabolic rate per $kg^{3/4}$ in each series can be approximated by a straight line. These

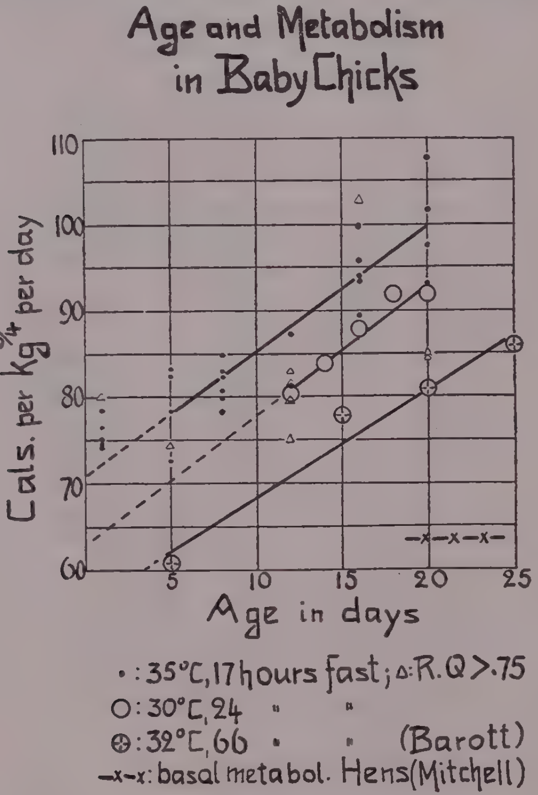

FIG. 1.

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[4] Barott, H. G., *et al.*, *J. Nutr.*, 1938, **15**, 150.

lines were calculated by the method of least squares. The results at one day of age have been omitted from this calculation since they do not represent fasting katabolism on account of unabsorbed yolk as shown by Barott and his coworkers.[5]

The results of the 3 series are not directly comparable. They were obtained at different environmental temperatures, but more important than these temperature differences seem to have been the differences in the length of the fasting period prior to the respiration trial. Thus the metabolic rate of the chicks of the 35° series, that had fasted only 17 hours was higher than the rate of the chicks with longer fasting periods even though the environmental temperature for the longer fasting chicks was lower, which ordinarily tends to increase rather than decrease the metabolic rate.

The main point of interest in the comparison of the 3 series is the fact that despite the differences in the experimental conditions all 3 series show a similar effect of age on the metabolic rate per $kg^{3/4}$. In order to compare this effect in one series with that in another the metabolic rate per $kg^{3/4}$ in each series can be regarded as the sum of a theoretical rate at birth and an increment for each day increase in age proportional to the rate at birth. This formulation is expressed by the following equation:

$$B_a = B_o(1 + \alpha a)$$

in which B_a = metabolic rate per $kg^{3/4}$ at a days of age
B_o = " " " " " birth (extrapolated)
a = age in days
α = age coefficient

The age coefficient for the 3 series in Fig. 1 is 0.026 kilocalories per $kg^{3/4}$ per day for the 30° series, 0.021 for the 35° series, and 0.022 for Barott's results. The metabolic rate per $kg^{3/4}$ of fasting chicks up to 20 days of age increases thus approximately 2% of the extrapolated metabolic rate at birth, for each day increase in age. The corresponding figure for full fed chicks calculated from the data mentioned above[2] amounts to 1.5%.

This relation of age on metabolic rate cannot be extrapolated beyond the age of 25 days. A calculation based on Barott's results[4] shows a maximum of the metabolic rate per $kg^{3/4}$ at 25 days of age, and the corresponding metabolic rate of mature hens calculated from data published by Mitchell and Haines[6] amounts to only 63.8 kilocalories[1] per day per $kg^{3/4}$, which level is indicated on Fig. 1 for comparison.

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[5] Barott, H. G., *et al.*, *J. Nutr.*, 1936, **11**, 191.

[6] Mitchell, H. H., and Haines, W. T., *J. Agr. Research*, 1927, **34**, 927.

TABLE I.
Relation of Metabolic Rate per Unit of Body Size to Age in Baby Chicks.

Daily metabolic rate kilocalories	per kg (weight)		per kg$^{3/4}$		per kg$^{2/3}$ (surface)	
Environmental temperature °C	30	35	30	35	30	35
Hours fast prior to resp. trial	24	17	24	17	24	17
Coefficient of correlation	.79	.23	.97	.89	.98	.91
Linear Regression:						
Extrapolated metabolic rate at birth, kilocalories	156.0	178.6	61.5	70.6	45.8	52.2
Age coefficient: Increase in metabolic rate for each day increase in age, kcal	1.50	0.32	1.60	1.47	1.40	1.45
Age coefficient in % metabolic rate at birth, %	1.0	0.2	2.6	2.1	3.1	2.8
Standard deviation of single measurement from predicted value, kcal	4.2	5.6	1.3	4.2	0.9	3.7
Standard deviation in % metabolic rate at birth, %	2.7	3.1	2.1	6.0	2.0	7.1

Table I contains the essential statistics for the influence of age on the metabolic rate per unit weight and per unit surface area in comparison with that per kg$^{3/4}$. It shows that the metabolic rate of fasting chicks increases with increasing age even if it is expressed per unit of body weight. In the 30° series this increase was quite distinct (correlation coefficient 0.8) amounting to 1% per day. The corresponding age coefficient of the metabolic rate per unit body weight in the 35° series was only 0.2% and the correlation coefficient in this case was only 0.2. In this series the metabolic rate per unit weight can therefore be summarized irrespective of age. It is 182.4 ± 1.7 kilocalories per day per kilogram body weight and the coefficient of variation of a single value from this mean is only ± 4.3%. The metabolic rate per unit surface area (kg$^{2/3}$) in the 2 series in Table I increases approximately 3% of the extrapolated rate at birth for each day increase in age.

Summary. Fasting baby chicks from 5 to 20 days of age showed an increase in metabolic rate with increasing age. This increase was 0.2 to 1%[‡] per day for the metabolic rate per unit body weight, 2% per day for the metabolic rate per kg$^{3/4}$ and 3% for the metabolic rate per unit surface area. Baby chicks thus deviate systematically from the rule which is valid for the relation of body size and metabolism in mature homeotherms. This confirms an earlier deduction based on an observation on full fed baby chicks.

[‡] Per cent of the extrapolated rate at birth.

10019

Presence of Antithyrotropic Substance in Serum of Rats Injected Chronically with Rat Pituitary Extract.*

EVELYN ANDERSON AND HERBERT M. EVANS.

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It was reported by Katzman, Wade and Doisy[1] that female rats receiving chronic implantation of anterior lobes of the same species for as long as 9 months still possessed enlarged ovaries and that there was no evidence of the existence of gonadotropic- or thyrotropic-inhibitory substances in the plasma of these rats.

We have carried out an investigation with regard to the thyrotropic hormone which sufficiently parallels the method used by these workers so that we feel a comparison of results is justifiable. A group of rats were injected daily with an alkaline extract of rat anterior pituitary for periods of 18 to 122 days. Each rat received the equivalent of 1 or 2 adult pituitaries daily. The serum was collected from these animals at the time of autopsy. This was examined for antithyrotropic substance, using immature guinea pigs as the test animal. The animals were given 1 or 2 cc of serum daily for 4 days. On the second, third and fourth days, a standard alkaline extract of beef pituitary was injected, the total dose for the 3 days being equivalent to 6 adult rat pituitaries, which was the amount used by the workers referred to above. The O_2 consumption of the guinea pigs was measured daily during the 5-day test period. The animals were autopsied on the fifth day, which was 96 hours after the first injection of pituitary extract. The failure of the rise in O_2 consumption of test animals to the expected level with a standard dose of thyrotropic hormone when serum was given, has been taken as an indication of antithyrotropic activity of the serum. Our results are shown in Table I. It was found that 4 cc of rat serum had very little effect in preventing the rise in O_2 consumption, while 8 cc of serum caused a significant depression of the O_2 consumption. The St. Louis investigators did not use a dose of serum greater than 4 cc. This may explain their failure to obtain evidence of antithyrotropic activity.

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* Aided by grants from the Board of Research of the University of California and Rockefeller Foundation of New York City.

We wish to acknowledge assistance rendered by the Federal Works Progress Administration, Project No. 7787.

[1] Katzman, P. A., Wade, N. J., and Doisy, E. A., *Endocrinology*, 1937, **21**, 1.

TABLE I.
Inhibitory Effect of the Serum from Rats Injected Chronically with Rat Anterior Pituitary Extract Using O_2 Consumption in Guinea Pigs as the Test Method.

	No. of days donors injected	Donors' serum total dose cc	No. of test animals	% change in O_2 consumption
1. Standard dose thyrotropic hormone only			25	+25
2. Normal rat serum	—	8	10	+20
3. Serum from rats injected with rat pituitary extract	18	4	2	+24
4. Serum from rats injected with rat pituitary extract	35	4	4	+23
5. Serum from rats injected with rat pituitary extract	35	8	6	+10
6. Serum from rats injected with rat pituitary extract	39	8	1	+14
7. Serum from rats injected with rat pituitary extract	122	4	4	+18
8. Serum from rats injected with rat pituitary extract	122	8	2	+ 9
9. Serum from rats injected with beef pituitary extracts*	120	4	13	— 2

The test guinea pigs received a total dose of standardized extract of beef pituitary which was equivalent to 6 adult rat pituitaries in terms of thyrotropic activity.

*The donors in this series were injected with an alkaline extract of beef anterior pituitary. The daily dose had 10 times the thyrotropic activity of one adult rat pituitary.

It may well be that if larger doses of thyrotropic hormone from rat pituitaries had been injected over a prolonged period, a serum more potent in antithyrotropic activity would have been obtained. We have included in Table I the effect of serum from rats which had been injected with beef pituitary extracts for 120 days, receiving a daily dose of thyrotropic hormone equivalent to 10 rat pituitaries, which was 5 to 10 times the dose given the rats in the other series. Four cc of this serum completely inhibited the effect of the standard test dose of thyrotropic hormone.

Conclusion. The injection of rat pituitary extract into rats gives rise to an antithyrotropic substance in the serum of these animals. This was detected by the depressing effect of the serum upon the O_2 consumption of guinea pigs receiving a standard dose of thyrotropic hormone.

10020

Cultivation of the Virus of St. Louis Encephalitis.*

E. W. Schultz, G. F. Williams and A. Hetherington.

From the Department of Bacteriology and Experimental Pathology, Stanford University, California.

Syverton and Berry[1] reported the successful cultivation of the virus of St. Louis encephalitis (epidemic encephalitis, Type B) in a medium containing minced living embryonic mouse-brains. Later, Harrison and Moore,[2] confirmed this work and in addition reported success in propagating the virus on the chorioallantoic membranes of developing chicks. This paper is a confirmation of both these reports.

The methods followed in our studies were in all essential respects the same as those reported by our predecessors. The mouse-embryo method was the same as that employed by Syverton and Berry, with the exception that 0.4 cc of material was transferred serially to culture-wells containing 2.5 cc of Tyrode's solution, 0.5 cc of normal rabbit serum and 0.5 cc of finely minced embryonic brains in Tyrode's solution (five, 14-day embryo-brains in 10 cc Tyrode's). Both the Li and Rivers[3] collar-flasks and 1 ounce flat-sided screw-cap bottles were used as receptacles for the medium. In propagating the virus on chorioallantoic membranes we initially followed the procedure (work carried out in 1936) described by Woodruff and Goodpasture,[4] and later a modification of this technic introduced by Burnet.[5] One-tenth cubic centimeter of a 10% suspension of the freshly harvested membrane in Tyrode's was inoculated in making the transfers. Ten- to 14-day eggs were employed. Webster's virus, strain No. 3, was used throughout.

The embryonic mouse-brain cultures of the virus were carried through 16 passages, with a final titer of 10^{-8}. From the 6th passage on, the virus was also carried on 2 additional media: (a) minced adult brain in place of embryonic brain, (b) minced adult brain without serum; the difference in volume was made up with

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* These studies were supported by the Mary Hooper Somers Fund for Filterable Virus Research.

[1] Syverton, J. T., and Berry, G. P., *Science*, 1935, **82**, 596.

[2] Harrison, R. W., and Moore, E., Proc. Soc. Exp. Biol. and Med., 1936, **35**, 359; *Am. J. Path.*, 1937, **13**, 361.

[3] Li, C. P., and Rivers, T. M., *J. Exp. Med.*, 1930, **52**, 465.

[4] Woodruff, A. M., and Goodpasture, E. W., *Am. J. Path.*, 1931, **7**, 209.

[5] Burnet, F. M., Med. Research Council, Special Rept. Series, No. 220, 1936.

Tyrode's solution. In both cases the virus was carried to the 12th passage (6 passages on adult brain), but in both it disappeared by the 14th passage.

A 7th-passage culture in embryonic mouse-brain after storage at 37.5°C for 35 days, caused fatal infection in mice.

On chorioallantoic membranes the virus was carried in one series through 15 passages; in another, through 16 passages, and in a third series through 22 passages. The first series was run by the shell-flap technic of Goodpasture, *et al.*,[4] the last 2 series by the artificial air-sac technic of Burnet.[5] In the first series transfers were made with 0.1 cc of a 1:5 suspension of the previous membrane; in the second and third 0.1 cc of a 1:10 suspension was inoculated. In the first 2 series the inoculated membranes at no time proved infectious in dilutions above 10^{-1}. In the third series it was 10^{-1} at the 5th passage, 10^{-2} at the 11th passage, 2×10^{-3} at the 17th passage and 2×10^{-4} in the 22d passage. This is considerably above that previously reported. Titration of the virus in brains of infected embryos (age 13 to 16 days) in the 17th passage was 2×10^{-4}. This suggests that the virus proliferates more actively in the brain than in the chorioallantoic membrane.

Changes similar to those previously described[2] were observed in chorioallantoic membranes. In the gross these ranged from a faint whitish stippling to a diffuse thickening with a certain amount of opacity, hyperemia, and hemorrhage. These changes were most evident after the third day, at which time foci of necrosis began to appear in the thickened areas. These increased in extent and death of the embryo occurred between the sixth and seventh day. Microscopically the membranes showed foci of ectodermal (sometimes endodermal) hyperplasia, vacuolation and necrosis of epithelial cells, and a purulent surface-exudate. The mesoderm often showed edema, polymorphonuclear-cell infiltration, and occasionally some increase in fibroblasts. The cytoplasm of some of the ectodermal cells presented small, round basophilic (sometimes eosinophilic) bodies of varying size. As D'Aunoy and Evans[6] have recently pointed out, certain of these changes, including the inclusion-bodies, may appear in chorioallantoic membranes under natural conditions. These changes, however, tend to be much more prominent in virus-inoculated membranes than in normal membranes and are supplemented by ectodermal necrosis and polymorphonuclear-cell infiltration.

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[6] D'Aunoy, R., and Evans, F. L., *J. Path. and Bact.*, 1937, **44**, 1937.

10021

Appearance of Vacuolated Cells in Hypophysis of *Triturus torosus* Following Bilateral Thyroidectomy.*

CLIFFORD GROBSTEIN. (Introduced by B. M. Allen.)

From the Department of Zoology, University of California at Los Angeles.

This paper is a report of studies made upon the morphological response of the anterior lobe of the hypophysis following bilateral thyroidectomy in the salamander, *Triturus torosus*. The work was undertaken as a prelude to physiological studies upon amphibia designed to throw light upon the possibility of a reciprocal action between the thyroid and hypophysis. It seemed necessary to determine first whether or not the anterior lobe of a typical amphibian shows morphological changes after thyroidectomy such as have been reported by numerous investigators in the mammalia. (See Severinghaus[1] for bibliography.)

The technic of thyroidectomy was essentially that recommended by Taylor.[2] Darkening of the integument[2, 3] occurred usually some 15 to 25 days after operation. Animals were killed by decapitation after periods ranging from 5 to 114 days.

All glands were fixed in Heidenhain's "Susa" (Sublimate-Trichloracetic Formalin) and imbedded by the dioxan method. Sections were cut at 5μ and stained in hematoxylin, acid fuchsin, and anilin blue. In several cases sections of the hypophyses from control and thyroidectomized animals were mounted on the same slide to ensure uniform treatment.

Glands from 8 controls and 33 operated animals were studied. It appears that the most conspicuous change in the anterior lobe of *Triturus torosus* following thyroidectomy is the development of vacuoles in the cytoplasm of certain basophilic cells. The exact classification of these cells has not yet been worked out but it is supposed that they are degranulated basophiles.

The vacuoles apparently first make their appearance in small clusters in one or 2 localized areas of the cytoplasm. They may be in close association with the nucleus or considerably removed from it, all on one side of the nucleus or scattered around it. From their distribution it is hard to see how the Golgi material could be concerned in their formation. Cells in this early stage of vacuolation tend to be more numerous after short periods of thyroidectomy but are regularly present also after longer periods.

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* I wish to express my sincere appreciation of the generous advice and encouragement given by Dr. Bennet M. Allen during the course of this study.

[1] Severinghaus, A. E., *Physiol. Rev.*, 1937, **17**, 556.

[2] Taylor, A., *J. Exp. Zool.*, 1936, **73**, 153.

[3] Adams, A. E., Kuder, A., and Richards, L., *J. Exp. Zool.*, 1932, **63**, 1.

The small vacuoles seem to coalesce fairly rapidly in later stages into larger ones of usually perfectly circular outline. These vary in size and number. In extreme cases they may occupy very nearly the entire cytoplasm and press in upon the nucleus. As many as 3 of these large vacuoles have been observed in a single cell.

The time of the beginning of the vacuolation process has been roughly estimated. The thyroidectomized animals may be segregated into 2 groups, 10 animals kept 20 days or less after operation, and 23 animals kept 26 days or more after operation. In the group of shorter duration only 2 animals developed vacuoles. Of these 2 one had been kept 15 days and the other 20 days after operation. In both cases the vacuolation was slight. Careful study of the glands of 8 other animals kept 13 to 20 days after thyroidectomy, as well as of 8 normal controls, revealed no significant vacuolation.

In the group of longer duration of thyroidectomy (26 to 114 days) only 4 out of 23 animals failed to show marked vacuolation. These 4 were also the only animals in the group which did not undergo the darkening of the integument characteristic of the thyroidectomized salamander.[4] On this basis, and because thyroid remnants were found in one of these animals, it is believed that they were incompletely thyroidectomized.

Thus it may be said that 19 animals kept longer than 26 days after operation and rendered sufficiently hypothyroid to induce darkening of the integument showed vacuolation, while 8 animals kept less than 21 days failed to show vacuolation and 2 only bare beginnings. It is concluded that the vacuolation process in the anterior lobe first becomes evident about 3 weeks after thyroidectomy.

A second change has been noted in these same basophilic cells in 8 of the 19 glands which showed cytoplasmic vacuolation. The nuclei of certain of these cells, never more than 4 or 5 in a section and usually less, develop small clear areas in the midst of the chromatin material. In later stages this area enlarges and becomes basophilic, frequently with a mass of heavily basophilic material at the center. The phenomenon has all the appearances of a disintegrative process.

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[4] Adams, A. E., and Grierson, M. C., PROC. SOC. EXP. BIOL. AND MED., 1932, **30**, 341.

This nuclear change has been observed in cells both with and without cytoplasmic vacuoles. It has never been observed in an acidophile nor in the basophiles of a non-vacuolated gland, whether control or following a short period of thyroidectomy. It seems highly probable that there is some correlation between the nuclear change and the cytoplasmic vacuolation and that the former, like the latter, is a characteristic response to thyroidectomy in Triturus.

10022

Choline and Pancreas Extract on Fatty Livers and Ketosis Due to Ant. Pituitary Extract.

EATON M. MACKAY AND RICHARD H. BARNES.
From the Scripps Metabolic Clinic, La Jolla, California.

These experiments were carried out when there was reason to believe that the pancreas extract described by Dragstedt, *et al.*,[1] under the name of lipocaic was a new fat metabolizing hormone. Recent developments[2, 3] suggest that this substance owes its activity to the choline and protein content and somewhat diminish the original intent of this work. However, the data reported here are still of interest in connection with the further differentiation of factors influencing fat transport to and from the liver.

The albino rats used were removed from the stock diet, fasted for 24 hours, weighed and urine collections commenced. The administration of 2 cc of 5% NaCl per day to each rat insured good urine volumes. The rats comprising a given group through the 6 experiments were all treated simultaneously. The ketogenic anterior pituitary extract was prepared according to the method of Best and Campbell.[4] The rats in experiments 2, 3, and 5 received 4 cc per rat per day subcutaneously. The rats receiving choline hydrochloride were given 2 cc of 10% solution by stomach tube at the beginning of each 24-hour period of urine collection. The pancreas extract was prepared according to the method of Dragstedt, *et al.*,[1] and was ad-

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[1] Dragstedt, L. R., Van Prohaska, J., and Harms, H. P., *Am. J. Physiol.*, 1936, **117**, 175.

[2] Best, C. H., and Ridout, J. H., *Am. J. Physiol.*, 1938, **122**, 67.

[3] MacKay, E. M., and Barnes, R. H., PROC. SOC. EXP. BIOL. AND MED., 1938, **38**, 410.

[4] Best, C. H., and Campbell, J., *J. Physiol.*, 1936, **86**, 190.

TABLE I.

Grp	*Sex	Body weight		Liver weight			Liver fat		Ketone excretion in mg per sq. dem body surface per day			N excretion per sq. dem. body surface per day	‡Ant. pituitary extr.	§Pancreas extr.	Choline hydrochloride
		†Initial g	Final g	†Body surface cm²	Observed g	Per sq. dem. body surface g	%	Per sq. dem. body surface mg	1	2	3	mg	g	g	mg
Exp. 1—Controls, no treatment															
A	♀	175	154	354	4.87	1.37	4.57	63	2	6	12	29			
B	♂	175	161	356	4.48	1.25	4.07	51	8	9	4	32			
Exp. 2—Ant. pituitary extract															
A	♀	175	158	354	5.79	1.62	7.62	125	30	36	25	35	.41		
B	♂	179	169	360	5.35	1.48	5.31	80	47	25	20	31	.41		
Exp. 3—Ant. pituitary extr. and pancreas extr.															
A	♀	174	165	352	6.71	1.88	6.14	116	26	35	20	36	.41	8.7	
B	♂	179	177	361	5.82	1.61	5.05	81	44	28	16	37	.41	8.3	
Exp. 4—Pancreas extr.															
A	♀	176	161	354	5.12	1.43	3.25	46	1	0	1	34		8.6	
B	♂	180	168	362	4.65	1.28	4.94	64	5	2	2	30		8.3	
Exp. 5—Ant. pituitary extr. and choline															
A	♀	178	163	357	5.98	1.66	6.52	108	39	24	11	32	.41		57
B	♂	182	170	365	5.72	1.57	4.87	77	42	16	5	38	.41		55
Exp. 6—Choline															
A	♀	177	156	356	4.54	1.28	3.88	49	3	2	2	34			57
B	♂	182	166	365	4.53	1.24	3.78	46	4	2	2	34			54

*The female groups were composed of 5 rats and the male groups of 7 rats each.

†Body weight when the feeding of the special diet was commenced and from which the body surface is calculated.

‡Weight of desiccated anterior pituitary gland which the extract given represented.

§Weight of fresh pancreas equivalent to the extract used. See text for nature of extract.

Doses listed in last 3 columns are in relation to sq. dem. body surface per day.

ministered in a solution containing 20.36% total solids and 2.42% nitrogen at a rate of 1 cc per sq dcm body surface per day. Nitrogen determinations were made by the macro Kjeldahl method and ketone bodies were determined by Van Slyke's method. Liver "fat" was determined by saponification, acidification, and extraction with petroleum ether.

The data presented in Table I show that the development of a significant ketonuria is uniformly accompanied by a deposition of fat in the liver which ensues when the anterior pituitary extract is administered to a fasting rat. Although both choline and the pancreas extract may have caused a slight diminution in the liver fat content of the control rats neither of these agents had a significant influence upon the increase in liver fat produced by the anterior pituitary extract (Exps. 2, 3, and 5). The ketonuria of both the choline and pancreas extract treated control rats (Exps. 4 and 6) was very slightly but definitely less than that of the controls (Exp. 1). The pancreas extract had no effect whatsoever upon the ketonuria due to the anterior pituitary extract although it was measurably reduced by the choline. This is in contrast to the ineffectiveness of choline in influencing the ketonuria which occurs during fasting after the production of a fatty liver by dietary means.[5]

Summary. The deposition of fat in the liver of fasting rats due to certain anterior pituitary extracts is not influenced by the administration of choline or a pancreas extract called lipocaic. The ketonuria which accompanies these fatty livers is not affected by the administration of the pancreas extract but is slighly reduced by feeding choline.

10023

Classification of Streptococci from Cases of Endocarditis.

HELEN MORAN. (Introduced by M. S. Fleisher.)

From the Department of Bacteriology, St. Louis University, St. Louis, Mo.

Sherman[1] recently offered a method of classifying the streptococci based mainly on physiological* tests which include growth at

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[5] Deuel, H. J., Jr., Murray, S., Hallman, L. F., and Tyler, D. B., *J. Biol. Chem.*, 1937, **120**, 277.

[1] Sherman, James M., *Bact. Rev.*, 1937, **1**, 1.

* The term physiological includes all tests other than fermentative tests.

10°C and at 45°C, growth in the presence of 6.5% NaCl or in the presence of 0.1% methylene blue, liquefaction of gelatin, and reaction in litmus milk.

It was the purpose of the study here reported to investigate the biochemical behavior of streptococci isolated from cases of endocarditis and to attempt to correlate their reactions in Sherman's tests with the reactions of the salivary streptococci or of the enterococci. The organisms investigated consisted of 20 strains isolated from the blood of cases which were clinically diagnosed as cases of endocarditis. These strains were submitted to us through the kindness of various hospitals throughout St. Louis and other cities of the United States. All organisms were found to be bile-insoluble.

Table I shows the reactions of the endocarditis-organisms in the physiological tests which Sherman considers to be of primary differential value. Twelve of the organisms tested are similar to *Streptococcus salivarius* according to Sherman's tests, 3 correspond to *Streptococcus fecalis* and 2 to *Streptococcus zymogenes*. Only in the case of the strain labeled Coffman is there any variation from the rule. This strain resembled *Streptococcus fecalis* in other ways but did not reduce the litmus of litmus milk until after the milk had been clotted. However, the other strains which resembled *Streptococcus fecalis* gave the typical reaction in milk, *i. e.,* reduction of the litmus before coagulation. The strains which resembled *Streptococcus zymogenes* showed the strong reduction which is typical for these organisms. Peptonization also occurred, additional evidence of their property of proteolysis.

Three strains, which have been designated as the R endocarditis-strains, produced an alkaline reaction in milk and thus failed to correspond to any of the hemolytic and non-hemolytic streptococci thus far known. These organisms were peculiar in other characteristics. In morphology, they tended to become quite rodlike and to form very short chains upon cultivation in the laboratory, although on first isolation, they appeared to be typical streptococci.

Many workers, previous to Sherman, based their classifications on fermentative reactions. Sherman considers these reactions to be too variable to use as a basis for the classification of the streptococci and considers the fermentative tests among those of secondary differential value.

The streptococci from endocarditis were tested fermentatively. In Table II is shown the number of strains fermenting the various sugars, arranged by groups as defined by physiological reactions shown in Table I. It was observed that the organisms that resem-

TABLE I.

Physiological Reactions of Endocarditis-Streptococci.

Strains	Hemolysis	Growth at		Growth in presence of		Gelatin Liquefied	Litmus Milk
		10°C	45°C	6.5% NaCl	0.1% M.B.		
Salivary Type							
B 9898	—	—	—	—	—	—	A (8)
2867	—	—	+	—	—	—	ACl + R (4)
2868	—	—	+	—	—	—	ACl (8)
2869	—	—	+	—	—	—	ACl + R (2)
Landt	—	—	—	—	—	—	A or — (8)
Levy	—	—	—	—	—	—	A (8)
2834	—	—	+	—	—	—	ACl + R (3)
Cushing	—	—	+	—	—	—	A (8)
Lilly	—	—	—	—	—	—	A (8)
Beahle	—	—	+	—	—	—	ACl + R (3)
Poole	—	—	+	—	—	—	ACl + R (4)
Barrett	—	—	—	—	—	—	A (8)
Fecalis Type							
Coffman	—	+	+	+	+	—	R (2) ACl (2)
8911	—	+	+	+	+	—	R (1) ACl (2)
V_1	—	+	+	+	+	—	R (1) ACl (2)
Zymogenes Type							
Dog 4	+	+	+	+	+	+	ACl, R, P (1)
V_2	+	+	+	+	+	+	ACl, R, P (1)
R Type							
2047	—	—	+	—	—	—	Alkaline (8)
Falkner	—	—	+	—	—	—	,, (8)
Cl 4395	—	—	+	—	—	—	,, (8)

A = Acid. ACl = Acid Clot. R = Reduction. P = Peptonization. Nos. in parentheses = No. of days.

TABLE II.

Number of Strains Fermenting Various Carbohydrates Arranged According to Groups.

	Maltose	Sucrose	Lactose	Mannite	Salicin	Raffinose	Inulin	Sorbitol	Trehalose	Esculin
Salivarius Type, 12 strains	12	12	12	0	7	3	2	0	7	3
Fecalis Type, 2 strains	3	3	3	2	3	0	0	2	3	3
Zymogenes Type, 3 strains	2	2	2	2	2	0	0	2	2	2
R Type, 3 strains	3	3	3	3	0	0	0	0	0	0

bled the *Streptococcus salivarius* in their physiological reactions, closely resembled the salivary streptococci in their fermentative reactions. The percentage of these which fermented salicin, raffinose, and inulin was somewhat lower than that given by Sherman. The 5 streptococci from endocarditis, which resembled the enterococci in their physiological reactions, also gave fermentative reactions typical of the enterococci. The Coffman strain again varied somewhat by its inability to ferment mannite or sorbitol.

The R strains gave peculiar reactions in the sugars. They produced acid very slowly in maltose, sucrose and lactose, and failed to ferment any of the other sugars. In this way, they resembled a possible fermentative type of *Streptococcus salivarius*.

These results indicate that, in their physiological and fermentative reactions, the streptococci of endocarditis resemble one of two groups, the salivary streptococci or the enterococci. These results suggest that the streptococci concerned in endocarditis may be of endogenous source and that the source may be either the upper respiratory or the gastro-intestinal tract.

10024

Reflux into the Major Pancreatic Duct During Cholangiography.

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During the past ten years at the University Hospital, studies have been made of the biliary tract by means of cholangiography. The first cholangiogram of this series made in November, 1928, drew attention to the interesting phenomenon of reflux of contents of the common bile duct into the major pancreatic duct. In a patient having a persistent biliary fistula due to a stricture at the ampulla the major pancreatic duct was visualized on X-ray examination.

The cholangiograms were made by injecting radio-opaque solution into biliary fistulæ or more often into T-tubes placed in the common bile duct at the time of operation. One X-ray picture was taken immediately, followed by others at intervals to determine the emptying of the duct system.

In 91 patients 203 cholangiograms were made. A reflux of the radio-opaque solution into the duct of Wirsung was demonstrated in 21 cases (23%). This finding was not constant in the same individual since in 79 cholangiograms made in these 21 cases the reflux was seen 36 times. However, in one patient filling of the duct of Wirsung occurred reglarly in 6 cholangiograms made over a 7-months period. That it may be difficult to demonstrate in an individual case is indicated by its occurrence in but 1 of 10 cholangiograms made over a period of 2 years in one case and 4 years in another case.

In 3 cases in which a reflux occurred, stones in the common bile duct were present and in 2 other cases a high grade stricture was noted. The remaining 16 cases presented varying degrees of spasm at the ampulla. It is obvious that a stone impacted at the ampulla of Vater is not a necessary or even common factor in conversion of the ducts into a continuous channel to allow reflux of bile into the duct of Wirsung.

Doubilet[1] found the occurrence of reflux into the pancreatic duct from the common bile duct in about one-fifth of his cases and considered spasm at the sphincter ampullæ as the causative agent.

In a personal communication, Kreilkamp and Boyden[2] record the presence of well developed sphincter ampullæ in 2 out of 12 maceration specimens of the major papilla. This incidence agrees with the frequency of reflux into the major pancreatic duct and suggests a spasm of the sphincter ampullæ as the etiologic factor.

Studies by McGowan, Butsch, and Walters[3] and Doubilet and Colp[4] have shown that morphine in the usual therapeutic dosage frequently will produce spasm of the sphincter of Oddi, thus increasing the intraductal biliary pressure. In one case in this series the major pancreatic duct was visualized on cholangiography following the injection of morphine whereas no reflux was demonstrable on 3 previous examinations.

Conclusions. 1. Reflux of contents of the common bile duct into the duct of Wirsung occurred in 21 of 91 cases (23%). 2. Such reflux is not dependent on obstruction at the ampulla due to stone or stricture but is probably more often due to spasm at the ampulla.

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[1] Doubilet, H., personal communication.

[2] Kreilkamp and Boyden, personal communication.

[3] McGowan, Butsch, J. M., and Volterse, W., *J. A. M. A.*, 1936, **106**, 2227.

[4] Doubilet, H., and Colp, R., *Surg., Gynecol. and Obstetrics*, 1937, **64**, 622.

10025

Influence of Prontosil-soluble on Beta Hemolytic Streptococci Growing in Tissue Culture Media.*

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On the basis of *in vitro* experiments with the hydrochloride of 4′ sulfonamide-2, 4 diaminoazobenzol (Prontosil) and other sulfonamide compounds, Levaditi and Vaisman[1] concluded that certain of these drugs neutralized streptococcal hemolysin and leukocidin.

Meyer[2] reported that Prontosil shows some neutralizing power for streptococcal hemolysin. He also states that "Prontosil" added to serum-broth "... prevents the production of toxins." We are not certain whether Meyer's conclusions should be interpreted as applying to Prontosil or Prontosil-soluble, *i. e.*, the disodium salt of 4′ sulfamidophenyl-2 azo-7 acetylamino-1 hydroxynaphthalene 3, 6 disulfonic acid, (or both). He lists both in his general statement on chemotherapy but does not refer to Prontosil-soluble again, although some of his expressions in the tables might be taken to imply that he was using Prontosil-soluble.

Recently Gross, Cooper, and Lewis[3] investigated the hemolysin-neutralizing power of a number of drugs including Prontosil-soluble. Of the related compounds, *i. e.*, sulfanilamide, Prontosil and Prontosil-soluble, the latter was the only one showing any significant neutralizing power.

With a view to determining the influence of Prontosil-soluble on streptococcal hemolysin under experimental conditions which are as physiological as can be attained *in vitro*, we have studied its effect on the hemolysing power of colonies of β streptococci growing in tissue-culture medium containing the drug (concentration 1:1000).

The culture-clots were made of 1 part autogenous heparinized rabbit-plasma and 2 parts autogenous serum extract of 6 day chick embryos. The extract was inoculated with suitable dilutions (to make 10^{-5} to 10^{-8} in the final medium) of a strain of β streptococcus recovered from a fatal septicemia in man. Sufficient rabbit's erythrocytes were added to the extract to make a 5% suspension in the final medium. Clotting takes place promptly on mixing the plasma and extract. Cultures are incubated at 37.5°C in Maximow moist chambers. Details of the methods have been described previously.[4, 5, 6]

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* Aided by a grant from the Committee on Scientific Research, American Medical Association, and technical assistance from the Works Progress Administration, project 5269, sub-project 237, and by the National Youth Administration. Prontosil-soluble kindly supplied by Winthrop Chemical Co.

[1] Levaditi, C., and Vaisman, A., *Compt. rend. Soc. de biol.*, 1935, **120**, 1077.

[2] Meyer, F., *Quart. Bull. Sea View Hosp.*, 1937, **2**, 148.

[3] Gross, P., Cooper, F. B., and Lewis, M., PROC. SOC. EXP. BIOL. AND MED., 1938, **38**, 275.

Colonies become visible in the cultures before lysis starts. Measurements of the colonial diameter and the zone of lysis can be made with accuracy at 60× magnification. The measurements are made with an eyepiece-micrometer previously standardized with a stage-micrometer (114 units = 1 mm). Photomicrographs can be made on Process film to show the diameter of both colony and zone of hemolysis.

The term "hemolytic index" is used to describe the hemolyzing power of a colony in relation to its diameter, *i. e.*, the diameter of the zone of hemolysis over the diameter of the colony.

Colonies of β streptococci growing in this medium containing 1:1000 Prontosil-soluble are qualitatively identical with colonies in the same medium without the drug.

Prontosil-soluble exhibits no bacteriostatic effect under these experimental conditions.

It is found, however, that colonies in the medium containing the drug show smaller zones of lysis in relation to their size, *i. e.*, lower hemolytic index.

In Table I an experiment is summarized showing the average size of the colonies and hemolytic zones in control and experimental sets. The hemolytic index is shown for both sets and the inhibition of the hemolytic zone (on the basis of diameter) for the experimental set.

It may be noted that hemolysin-production is continuous, once

TABLE I.

	Controls			Prontosil-soluble			
Time	Diam. colony	Diam. H zone	Hem. index	Diam. colony	Diam. H zone	Hem. index	% Hem. inhib.
18 hr	39.76	64.99	1.63	40.23	52.71	1.31	18.9
24 ,,	78.49	132.86	1.69	76.3	106.2	1.39	21.0
48 ,,	214.7	354.0	1.64	219.2	273.9	1.24	22.6
3 days	252.4	399.5	1.58	262.6	310.3	1.18	22.4

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[4] King, J. T., *Arch. f. Exp. Zellforsch.*, 1930, **9**, 341.

[5] King, J. T., *Arch. f. Exp. Zellforsch.*, 1931, **10**, 467.

[6] King, J. T., *Arch. f. Exp. Zellforsch.*, 1937, **20**, 208.

started, under these conditions. In other experiments it has been observed to continue into the 5th day.

In some experiments we have observed somewhat greater inhibition of lysis than that seen in the series summarized in Table I. We have never seen anything approaching complete inhibition of lysis, however.

The technic used measures the inhibition of hemolytic damage in relation to control preparations. It does not distinguish between reduction in the amount of hemolysin formed and subsequent neutralization.

The results are in harmony with those obtained by others using neutralization-tests on active culture fluids. If Meyer's conclusions were intended to apply to Prontosil-soluble, then our results are not in accord with his finding that hemolysin-formation is prevented when β streptococci are grown in presence of the drug.

It seems to us that tissue-culture clots holding erythrocytes in fixed position in relation to the developing colonies provide a means of registering quantitatively the activity of hemolysin as formed and under conditions as nearly comparable to those existing in the body as can be arranged *in vitro* at present.

Conclusion. Beta hemolytic streptococci growing in tissue-culture media containing Prontosil-soluble, 1:1000, produce hemolysin. The amount available for erythrocytic destruction is moderately reduced as compared with controls. This effect is not secondary to bacteriostasis.

10026

Response of Leukocytes to Colonies of Streptococci Growing in Tissue-culture Media.*

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In discussing the mechanism of action of sulfanilamide, Mellon and Bambas[1] state, " . . . the growth inhibition brought about by the indirect action of sulfanilamide predicates a suspension in the production of necrobiotic substances (leucocidins, etc.) by the streptococci. As a result, the phagocytes are probably mobilized in proportion to the suppression of the negative chemotactic substances."

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* Aided by a grant from the Committee on Scientific Research, American Medical Association, and technical assistance from the Works Progress Administration, project No. 5209, sub-project 237, and by the National Youth Administration.

[1] Mellon, R. R., and Bambas, L. L., *Med. Rec.*, 1937, **146**, 247.

Recently McCutcheon, Coman, and Dixon[2] studied the response of leukocytes to particles of kaolin and Lloyd's reagent and to a strain of β streptococci, with a view to determining whether or not a true negative chemotropism could be demonstrated. The leukocytes were suspended in plasma which was flooded over the particles or organisms to be studied. They observed a true negative chemotropic response to particles of kaolin and Lloyd's reagent, *i. e.,* the cells were repelled. The response to the strain of streptococci used was sometimes positive and sometimes negative.

In experimental animals infected with β streptococci it has been noted by Domagk,[3] Long and Bliss,[4] and others that phagocytosis is apt to be less marked in the controls than in the animals treated with sulfanilamide or derivatives. However, it has not been possible to demonstrate that these substances directly influence phagocytosis *in vitro* (Finklestone-Sayliss, *et al.;*[5] Osgood and Brownlee;[6] Coman[7]).

The theory advocated by Bliss and Long[8] and by Gay and Clark[9] postulates a reduction in toxic substances due to the bacteriostatic action of sulfanilamide but it is not assumed that negative chemotropism is the explanation for the reduced phagocytic activity shown by cells in untreated animals.

In the paper referred to above, McCutcheon, Coman, and Dixon point out that the terms "negative chemotropism" and "negative chemotaxis" have been very loosely used in the past and that either a true negative chemotropism (actual repulsion) or merely the absence of positive chemotropism would result in an essential failure of phagocytosis. It is evident that proper distinction has not always been made between that reduction in, or failure of, phagocytosis which is due to true negative chemotropism and that due to other causes.

In the case of the β streptococcus, it is well known that phagocytekilling substance or substances are produced. Infected, untreated controls show not only fewer phagocytic cells in exudates but also a leukopenia. The cells, especially the granulocytes, show degenerative changes. It becomes important, therefore, to determine whether, in addition to this general toxic depression of phagocytes, true negative chemotropism is a factor in producing the observed diminution of phagocytosis.

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[2] McCutcheon, M., Coman, D. R., and Dixon, H. M., *Arch. Path.,* in press.

[3] Domagk, G., *Z. f. Klin Med.,* 1937, **132**, 775.

[4] Long, P. H., and Bliss, E. A., *J. A. M. A.,* 1937, **108**, 32.

[5] Finklestone-Sayliss, H., Paine, C. G., and Patrick, L. B., *Lancet,* 1937, **233**, 792.

[6] Osgood, E. E., and Brownlee, I. E., *J. A. M. A.,* 1938, **110**, 349.

[7] Coman, D. R., *Arch. Path.,* in press.

[8] Bliss, E. A., and Long, P. H., *J. A. M. A.,* 1937, **109**, 1524.

[9] Gay, F. P., and Clark, A. R., *J. Exp. Med.,* 1937, **66**, 535.

Tissue-cultures have been utilized in studying the response of rabbit's leukocytes to 2 strains of streptococci. Fragments of buffy coat from centrifuged blood were planted in clots composed of one part autogenous plasma (heparinized) and 2 parts chick-embryo extract made with autogenous serum. Methods have been described by one of us[10, 11, 12] previously. The tissue-extract was inoculated before mixing with the plasma with suitable dilutions (usually between 10^{-5} and 10^{-8}) of 18- to 24-hour broth cultures of the streptococcus. The dilutions were made in Tyrode's solution. The cultures were planted on 22 mm round cover-slips (volume approximately 0.15 cc). After clotting, the cultures were incubated (Maximow moist chambers) at 37.5°C.

Bacterial colonies usually become microscopically visible by the 6th hour and they grow rapidly in this medium. If the medium contains approximately 150,000 colonies per cc, growth is essentially complete at 12 hours. With 5 colonies or less per cc, growth may continue into the 5th day.

Cell-migration is prompt and rapid in this medium. As the edge of the dense field of migrating cells approaches and passes the growing colonies one has an excellent opportunity to observe the response of the leukocytes to the streptococci. After making observations on the living state, cultures may be fixed in Carnoy and stained in dilute Delafield's hematoxylin and mounted as whole preparations, or fixed in Zenker-formol for serial sections. Good differential staining of sections is obtained with Dominici.

Two strains of streptococci have been used in these studies: (a) a β strain recovered from a case of meningitis which recovered on treatment with sulfanilamide. This strain was very sensitive to sulfanilamide in tissue-culture media; (b) a β strain recovered from a fatal septicemia in man. This strain was also sensitive to sulfanilamide *in vitro*.

Observations on the living cultures during the first 24 hours

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[10] King, J. T., *Arch. f. Exp. Zellforsch*, 1930, **9**, 341.

[11] King, J. T., *Arch. f. Exp. Zellforsch*, 1931, **10**, 467.

[12] King, J. T., *Arch. f. Exp. Zellforsch*, 1937, **20**, 208.

showed that the advancing edge of the leukocytic migration-zone presented a finely or coarsely granular appearance according to whether the bacterial colonies were small and numerous or larger and fewer in number. This finding is in marked contrast to the edge of the migration-zone in sterile cultures where the edge is smooth and the distribution of cells quite uniform.

Examination of fixed and stained *in toto* mounts showed that the bacterial colonies were densely infiltrated with leukocytes. Colonies that are just being approached by the leukocytes show infiltration predominantly on the side from which the cells are approaching while colonies that have been passed by the edge of the migration zone are more uniformly infiltrated.

Study of stained serial sections showed that phagocytosis was taking place actively in each instance.

Using the first strain described, special attention was given to the effect of varying the number of colonies. It was noted in cultures containing a larger number of streptococci that activity of granulocytes was depressed earlier. In one series stained as *in toto* mounts at 24 hours, 2 dilutions of the same bacterial culture were used for inoculating the tissue-culture medium. The dilutions in the final medium were 1:1200 and 1:120,000. In the higher concentration there were practically no living cells in the periphery of the migration-zone at 24 hours while in the lower concentration the toxic depression was not so marked. As would be expected, the width of the migration-zone varied directly with the dilution of bacteria. The average maximal width of the zone was 227.5† for the 1:1200 dilution and 360 for the 1:120,000. These values are taken to indicate the activity of the granulocytes since the periphery of the migration-zone contains few non-granular elements.

There is evidence suggesting that the non-granular elements are less sensitive to the toxic substance formed by the streptococci than are the granulocytes. This question is being investigated further.

Conclusions. Observations made on 2 strains of β streptococci show that rabbit's granulocytes exhibit positive chemotropic response to both strains. There is a progressive toxic depression of phagocytosis varying with the number of organisms present. For the strains investigated true negative chemotropism does not play a part in the depression of phagocytosis.

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† 114 units = 1 mm.

10027 P

Occurrence of Shock Disease Among Young Snowshoe Hares.*

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The term, shock disease,[1] has been introduced to describe a disease of snowshoe hares which is associated with the periodic die-off of these animals in the wild. Hares afflicted with this condition have a disturbance of carbohydrate metabolism resulting from liver degeneration. Shock disease has been observed among adult hares in their natural environment, and its presence in hare populations has been demonstrated during the winter months by trapping and holding animals in captivity. Hares taken from certain areas succumbed rapidly from the shock of unnatural conditions, and death was usually accompanied by an extremely low blood sugar level.

The recognition of shock disease among adult hares during winter months raised the question of whether this disease occurs in young hares during the warm months. To gain this information, adult hares were placed in a fenced area of natural habitat in the spring of 1936 and, together with the young subsequently born, were kept under close observation throughout the summer. The fenced area, called "Range A", consisted of 1.7 acres enclosed with wire of ¾" mesh to prevent the escape of young hares. Forty-one females were introduced into this range, most of them in the latter part of April. Six males were entered in April, 3 in May, and 1 in June. Natural forage was available throughout the study, with daily supplemental feeding of carrots, oats, cut aspen, and willow. The young were born in 3 groups: 36 from May 3 to 18; 51 from May 28 to June 26; and 28 between July 1 and 17. Most of them were banded with ear tags at an age of 3 to 5 days, while still in the nest.

Losses, due principally to shock disease, were heavy among both adult and young hares in this range. At the conclusion of the investigation, on November 9, 20 of the 41 females and 8 of the 10 males had died. Only 8 young, 7% of those born, survived the study. The carcasses of 82 young hares were recovered for examination.

During the course of the experiment, diagnoses of shock disease

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* From the Cooperative Wildlife Disease Investigation of the University of Minnesota, the Minnesota Department of Conservation, and the Bureau of Biological Survey.

[1] Green, R. G., and Larson, C. L., *Am. J. Physiol.*, 1937, **119**, 319.

were made on a basis of blood sugar determinations in addition to recognition of symptoms and postmortem findings.

Adult hare, band No. 4274, was found sick with typical shock symptoms in Range A on September 17. This male hare had been released in the pen on May 26 and developed shock 114 days after entrance. When discovered, it was taken into the field laboratory and bled. Its blood sugar content was less than 10 mk per 100 cc, and necropsy findings were characteristic of shock disease.

The occurrence of shock disease in young hares was rigidly demonstrated by studies on hare No. 4237. This animal was born on May 6, 1936. On July 1 it was captured in order that a sample of blood might be taken. Its blood sugar level was normal. The hare was returned to the range, and at 9:30 the following morning was found huddled up, indifferent to the approach of an observer. At 11:15 it was lying on its side, too weak to assume a normal posture, and when picked up and carried into the laboratory, it developed convulsions. Its blood contained 3 mg sugar per 100 cc. This hare, born in the range and living under essentially natural conditions, died from shock disease 57 days after birth.

Many young hares that had never been handled except during the brief process of ear-banding, died of shock disease, typical both as to symptoms and postmortem findings. The second group of hares born, a total of 51, suffered a mortality of 25 animals or 49% when the hares were 5, 6, and 7 weeks old.

These studies were made during the second year in which Range A was used to hold adult and young hares. As the mortality during the second year was much increased over that of the first year, it would seem that the factor causing shock disease became accentuated with repeated use of the same area for holding concentrated populations. The experiment not only establishes the occurrence of shock disease during summer months but also demonstrates that young hares may die of this disease within 6 weeks of birth.

10028

Spermatogenesis in Immature Hypophysectomized Rats Injected with Androgens.

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Naturally occurring or synthetic androgenic substances injected into adult rats shortly after hypophysectomy will cause varying degrees of tubular maintenance in the testes,[1-5] but the interstitial cells are not prevented from undergoing atrophy.[2-5] After tubular atrophy in hypophysectomized rats has occurred, it has not been possible to restore spermatogenic activity by means of androgens.[2-5] Maintenance of spermatogenesis in hypophysectomized rats by androgens has not as yet been satisfactorily explained. The possibility that androgens have only an indirect effect on the germinal epithelium, scrotal maintenance being of prime importance,[3, 5] has not been accepted by Nelson and Merckel.[4] Incontestable evidence that male hormones have a direct or only an indirect effect on spermatogenesis is still lacking.

It has seemed of importance in view of our present lack of understanding with regard to the rôle played by androgens in spermatogenesis to study the effects of male hormones in immature hypophysectomized rats. This has seemed especially important since it has been shown that bull testis extracts[6] and androsterone[7] cause marked injury to the testicular tubules of young rats. The purpose of this investigation was to discover whether spermatogenesis would occur in immature hypophysectomized rats injected with androgenic substances. As we have never observed sperm heads, as described by Moore,[8] earlier than the 34th day of life, we selected animals much younger than this to rule out the possibility that sperm

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[1] Walsh, E. L., Cuyler, W. K., and McCullagh, D. R., *Am. J. Physiol.*, 1934, **107**, 508.

[2] Nelson, W. O., and Gallagher, T. F., *Science*, 1936, **84**, 230.

[3] Cutuly, E., McCullagh, D. R., and Cutuly, E. C., *Am. J. Physiol.*, 1937, **119**, 121.

[4] Nelson, W. O., and Merckel, C. G., Proc. Soc. Exp. Biol. and Med., 1937, **36**, 825.

[5] Cutuly, E., McCullagh, D. R., and Cutuly, E., *Am. J. Physiol.*, 1938, **121**, 786.

[6] Moore, C. R., and Price, D., *Am. J. Anat.*, 1932, **50**, 13.

[7] Moore, C. R., and Price, D., *Endocrinology*, 1937, **21**, 313.

[8] Moore, C. R., *Am. J. Anat.*, 1936, **59**, 63.

TABLE I.

				Daily injections				Autopsy data				
									Wt. (g)			
Litter	Rat No.	Age at start of expt (days)	Total No. days hypophysectomized	Substance	Amt (gamma)	Oil (cc)	Total No.	Age (days)	Testes	Seminal vesicles	Adrenals	Histology of testis tubules†
1	39	27	7	TP‡	50	1/20	6	34	.20	.016	.011	Atrophic
	40	27	7	Hypophysectomized control				34	.19	.006	.012	,,
	41	27	—	Normal control				34	.80	.011	.027	IV
2	42	27	12	TP	50	1/20	11	39	.14	.047	.009	Atrophic
	43	27	21	TP	50*	1/20	20	48	.13	.427	.007	,,
	45	27	21	TP	50*	1/20	20	48	.11	.655	.008	,,
	44	27	12	Hypophysectomized control				39	.11	.005	.008	,,
	46	27	—	Normal control				39	1.21	.022	.029	V
3	47	28	16	TP	100	1/20	15	44	.16	.305	.009	Atrophic
	48	28	16	TP	200	1/20	15	44	.23	.541	.016	,,
	49	28	16	TP	300	1/20	15	44	.29	.643	.008	,,
	51	28	—	Normal control				44	1.47	.040	.031	V
4	71	29	—	Reference control				29	.46	—	.025	II
	66	29	8	TP	2000	1/5	7	37	.54	.231	.018	IV
	67	29	8	TP	2000	1/5	7	37	.59	.196	.015	V
	68	29	8	DA‡	2000	1/5	7	37	.42	.033	.012	II
	69	29	8	Hypophysectomized control				37	.21	.008	.010	Atrophic
	71	29	—	Normal control				37	.83	.020	.022	IV
5	61	30	14	DA	2000	1/5	14	44	.63	.099	.010	V
	60	30	14	TP	2000	1/5	14	44	.59	.592	.014	V
	64	30	14	TP	2000	1/5	14	44	.62	.646	.016	V
	62	30	Normal	TP	2000	1/5	14	44	1.22	.785	.038	V
	59	30	14	Hypophysectomized control				44	.22	.013	.009	I
	65	30	—	Normal control				44	1.35	.053	.025	V

†I to V denote most advanced stage of spermatogenesis seen in tubules. I—primary spermatocytes; II—secondary spermatocytes; IV—sperm heads; V—spermatozoa.

‡TP—testosterone propionate and DA—dehydroandrosterone acetate.

*The last 9 injections were increased to 300 gamma daily.

heads or spermatozoa were present in the testes prior to the beginning of the experiment.

As will be seen in Table I five litters of rats were used. Their ages ranged from 27 to 30 days. In each litter one animal was set aside as a normal control; in 4 of the litters there was also a hypophysectomized control. Litter 4 contained a reference control which was killed at the start of the experiment. Hypophysectomized rats (and one normal rat in litter 5) were injected subcutaneously with testosterone propionate or dehydroandrosterone acetate.* Autopsies were performed 24 hours after the final injection, except in litter 4, where they were performed 48 hours after the last injection. The daily doses ranged from 50γ to 2 mg. Data such as the amount of oil (sesame) vehicle, length of experiment, and autopsy findings will be found in the table. It is realized that the series of animals is small. However, the appearance of sperm heads or spermatozoa in testicular tubules previously lacking such structures is a readily demonstrable and unequivocal criterion. The completeness of all the hypophysectomies was checked by a critical study of serial sections of the pituitary capsules which had been carefully dissected away from the sella. In addition the pieces of gland aspirated into the suction tube at the time of operation were always recovered to see if the fragments (usually 3) could be pieced together to form the whole gland. Finally, no hypophysectomized animal was included in the table unless its autopsy body weight was less than its initial weight, or unless the increase in autopsy body weight was far less than that in normal controls.

The data on litters 1, 2, and 3, in Table I show that testosterone propionate in daily doses as high as 300γ failed to have any effect upon the testes, even though the larger doses produced marked stimulation of the seminal vesicles. Testosterone propionate at the 50γ level caused scarcely any noticeable scrotal stimulation; and even at the 300γ level the scrotal response was weak. This was unexpected, as other observations[9, 10] have indicated that the scrotum is very sensitive to stimulation by male hormone produced by the testes. Since the scrotal turgescence induced by 300γ of testosterone propionate was slight, and despite the fact that the accessories were tremendously enlarged; it was decided to inject male hormone at much higher levels. This was done in litters 4 and 5.

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* We are indebted to Dr. Erwin Schwenk of Schering Corporation, Bloomfield, N. J., for the androgenic substances.

[9] Cutuly, E., and Cutuly, E. C., Proc. Soc. Exp. Biol. and Med., 1937, **37**. 477.

[10] Cutuly, E., and Cutuly, E. C., *Endocrinology*, 1938, **22**, 568.

The following results were obtained in litter 4. Two milligrams of testosterone propionate were injected daily for 7 days into rats hypophysectomized when 29 days of age. This amount of hormone caused an increase in testis weight beyond that in the reference control, but not equal to that in the normal control. However, the testis tubules of the injected animals, as well as those of the normal control, contained sperm heads or spermatozoa, while those of the reference control contained only secondary spermatocytes. Dehydroandrosterone acetate at a 2 mg level was unable to advance the condition of the testes beyond that in the reference control; but it did prevent the testes from undergoing the profound atrophy seen in the hypophysectomized control. In litter 5 both dehydroandrosterone acetate and testosterone propionate when given in daily doses of 2 mg for 14 days after hypophysectomy induced the formation of mature spermatozoa in the seminiferous tubules, although the testis weight was only about half that of the normal control. It is interesting to note that the normal animal in litter 5 which received 2 mg of testosterone propionate daily had testes which weighed just slightly less than those of the normal control. Histologically the testes of the injected normal animal showed perfectly normal tubules; but the interstitial cells of the testes were noticeably not as well developed as those in the normal control.

In all the hypophysectomized animals, treated or untreated, the interstitial cells were markedly atrophic, and this would account for some of the weight differences between the testes of these animals and those of the controls. Weight variations, however, were chiefly due to differences in the size of the tubules. This was indicated by measurements on tubules made with a micrometer eyepiece. For example, in litter 4 an average of 20 tubules for each testis measured showed that the diameter of the tubules of the normal control was 189.4μ, while that of rat numbers 66, 67, 68, and 69 was 167.9, 159, 161.8, and 119.5, respectively.

Of special interest was the finding that some of the hypophysectomized animals injected with testosterone propionate (rats Nos. 48, 66, 67, 60, 64) had adrenal glands which were not as small as might have been expected. Examination of histological sections of these glands showed them to be readily distinguishable from sections of normal adrenals, since they possessed typically shrunken cortices. The unexpectedly high weight of these glands was probably mostly due to variable quantities of lipoidal material in the cortex, since it has been demonstrated that the medulla does not change greatly in weight following hypophysectomy.[11] Although the adrenal cortex of rat 66 was characteristically shrunken, mitotic figures were seen in or near the glomerular zone. These observations tend to suggest (a) a possible direct or indirect action of male hormone on the adrenal cortex and (b) a possible connection between the adrenal cortex, male hormone, and spermatogenesis.

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[11] Cutuly, E., *Anat. Rec.*, 1936, **66**, 119.

It has been very difficult to reconcile the data showing that male hormones maintain spermatogenesis in adult hypophysectomized rats,[1–5] initiate spermatogenesis in ground squirrels,[12] fail to maintain spermatogenesis in hypophysectomized monkeys[13] and hypophysectomized guinea pigs,[14] and cause marked injury to the testis tubules in normal young rats.[6, 7] The present study shows that spermatogenesis can be induced in hypophysectomized rats with previously aspermatic testes. The relatively large dose necessary for this suggests that dosage is possibly responsible for the apparent discrepancies which have been mentioned. Size of accessory organs, as shown by this investigation, is certainly no criterion of the adequacy of the dose of androgen necessary to induce spermatogenesis.

It has already been pointed out that there is little correlation between the ability of an androgen to maintain testis tubules and its ability to stimulate the accessory sexual organs.[4, 5] The present findings not only confirm these reports, but they emphasize the fact that relatively enormous doses of the most potent androgen now available (testosterone propionate) are needed to cause a tubular response. It is difficult to understand why this should be so, especially when it is remembered that in the normal immature rat the testis tubules may develop sperm heads before the seminal vesicles show any appreciable secretory activity.[8] Because of this contrast between normal development and that brought about by a male hormone such as testosterone propionate, the following point seems worthy of mention. If the testis hormone (or hormones) produced by the interstitial cells normally has a stimulating effect on the tubules of the testis, then testosterone propionate is decidedly inferior to testis hormone in this respect. This inferiority on the part of an intensely strong androgenic substance may indicate (1) a real dissimilarity between it and interstitial cell hormone; (2) the inability of such a substance to express itself fully in a hypophysectomized animal, or the inability of organs to respond fully to it; (3) that it acts only indirectly on the spermatogenic elements.

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[12] Wells, L. J., and Moore, C. R., *Anat. Rec.*, 1936, **66**, 181.

[13] Smith, P. E., and Engle, E. T., mentioned by Scowen, E. F.

[14] Scowen, E. F., *Anat. Rec.*, 1938, **70**, 71 (suppl.).

Summary. Testosterone propionate and dehydroandrosterone acetate when administered daily at a 2 mg level to immature hypophysectomized rats induced sperm head or spermatozoon formation in the seminiferous tubules. Smaller doses were ineffective in this respect, even though they caused marked stimulation of the accessory organs. Testosterone propionate seemed in a few instances partially to prevent the adrenal cortex shrinkage which follows hypophysectomy; it is not known whether this effect was the result of a direct or indirect action by male hormone. An explanation which helps to reconcile the apparently discordant reports on the effects of androgens on spermatogenesis is suggested.

10029

Vitamin B_1 in Bacterial Metabolism.*

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Since the work of Peters[1] showing that the oxidation of pyruvic acid in pigeon-brain requires the presence of vitamin B_1, attention has been called to the function of this vitamin in the pyruvate-metabolism of yeast and bacteria. Lohmann and Schuster[2] have demonstrated that the co-enzyme of carboxylase, co-carboxylase, is diphosphorylated vitamin B_1. Lipmann[3] has reported that the phosphorylated vitamin is essential for the dismutation of pyruvate by an acetone preparation of *Bacillus acidificans longissimus.* In the same paper he states that the non-phosphorylated vitamin is without stimulating effect. Krebs[4] reports that no stimulation in the metabolism of pyruvates by *Staphylococcus aureus* could be obtained on the addition of vitamin B_1. However, Hills[5] has reported a marked stimulation in the pyruvate-metabolism of *Staph. aureus* grown in vitamin B_1-deficient media by the simple addition of crystalline vitamin B_1.

The purpose of the present investigation was to determine the

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* Supported in part by Industrial Science Research funds of Iowa State College.

[1] Peters, R. A., *Biochem. J.*, 1936, **30**, 2206.

[2] Lohmann, K., and Schuster, Ph., *Angew. Chem.*, 1937, **50**, 221.

[3] Lipmann, F., *Enzymologia*, 1937, **4**, 65.

[4] Krebs, H. A., *Biochem. J.*, 1937, **31**, 661.

[5] Hills, G. M., *Biochem. J.*, 1938, **32**, 383.

effect of the addition of free vitamin B_1 on the metabolism of various bacteria grown in B_1-deficient media,

Bacteria. The organisms employed were *Escherichia coli* (26), *Aërobacter indologenes* (23B), *Propionibacterium pentosaceum* (49W), and *Propionibacterium peterssonii* (11W). The propionic-acid bacteria were chosen for study since it has been shown by Tatum, Wood, and Peterson[6] that vitamin B_1 has an important rôle in the growth of these organisms. *A. indologenes* was cultured in an ammonium-sulphate, glucose, tap-water medium. The other organisms were grown in the basal medium of Tatum, *et al.*,[6] containing ethereal extract of yeast and hydrolyzed gelatin with and without the addition of crystalline vitamin B_1 in a final concentration of 10^{-8} g per cc.

The organisms were harvested by centrifuging after 60 hours of growth at 30°C. After one washing with distilled water, suspensions were made in distilled water containing 10% of cell-paste by volume. One-half cc of the suspension of propionic-acid bacteria and 0.25 cc of the *E. coli* and *A. indologenes* were employed in each of the experimental Warburg vessels according to Dixon.[7] The total volume in each vessel was 2 cc.

The pyruvic acid was redistilled twice at 59-63°C at 15 mm of mercury. Three mg of acid neutralized with sodium hydroxide were used in each cup. The buffer employed (1 cc in each cup) was M/5 monopotassium phosphate adjusted to pH 5.6 with sodium hydroxide.

All experiments were conducted anaërobically under an atmosphere of nitrogen.

Fig. 1 shows the stimulating effect of vitamin B_1 on the dismutation of pyruvate by both *P. peterssonii* (11W) and *P. pentosaceum* (49W). *P. peterssonii* (11W) grown on vitamin B_1-deficient media exhibits almost no activity. On the addition of 2 gammas of vitamin B_1, its activity is immediately enhanced. The effect of the vitamin on *P. pentosaceum* (49W) is similar, its activity being increased about 4 times. The addition of 2 gammas of the vitamin to *P. pentosaceum* (49W) cells grown in a concentration of 10^{-8} g of vitamin per cc increases its metabolic rate to almost that of the vitamin B_1-deficient cells to which 2 gammas of the vitamin has been added.

A. indologenes and *E. coli* although grown in vitamin B_1-deficient media were very active and showed no increase in the rate of dis-

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[6] Tatum, E. L., Wood, H. G., and Peterson, W. H., *Biochem. J.*, 1936, **30**, 1898.

[7] Dixon, Malcolm, *Manometric Methods*, Cambridge Univ. Press, 1934.

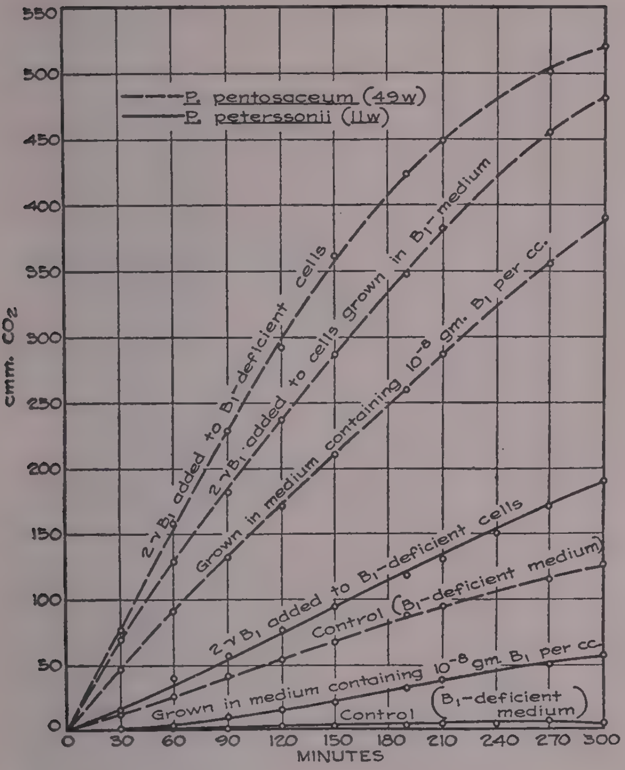

Fig. 1 EFFECT OF B_1 ON PROPIONIC ACID BACTERIA

similation of pyruvates on the addition of crystalline vitamin B_1. Since members of the Escherichia-Aërobacter group grow well in synthetic media, they are in all probability capable of synthesizing the vitamin as rapidly as it is required so the need for it cannot be demonstrated. Sunderlin and Werkman[8] reported the synthesis of vitamin B by *E. coli.*

On the other hand, propionic-acid bacteria are difficult to culture in synthetic media; one possible reason being their inability to synthesize the vitamin, therefore its need can be readily demonstrated.

The optimal concentration of the vitamin for the anaërobic breakdown of pyruvic acid is shown in Fig. 2. Under the conditions of our experiment, maximal activity is attained at a concentration in the vicinity of 0.5 gamma per 2 cc of liquid, equivalent to $2.5x10^{-7}$ g of vitamin per cc. Reference to Fig. 1 shows that the activity due

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[8] **Sunderlin, G., and Werkman, C. H., *J. Bact.*, 1928, 16, 1.**

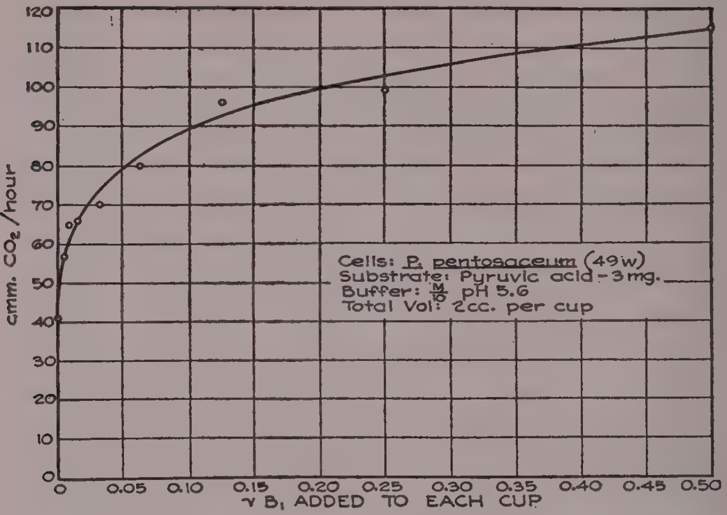

Fig. 2 EFFECT OF VARYING CONCENTRATIONS OF B_1 ON B_1-DEFICIENT CELLS

to 2 gammas of vitamin per cup does not greatly exceed that at ½ gamma per cup. In this respect it is interesting to compare this value with that of Tatum, *et al.*, for the optimal growth concentration of propionic-acid bacteria. They report a growth-optimum at a concentration of 0.5 gamma per 100 cc or 0.5×10^{-8} gammas per cc. This is 50 times less than the optimum concentration for anaërobic pyruvate-metabolism.

TABLE I.

Gammas vitamin B_1 added	0	.0039	.0078	.0156	.0313	.0625	.125	.25	.5
CO_2 evolved in mm^3/hr	41	57	65	66	70	80	96	99	115

Table I shows that the addition of 0.0039 gammas or 3.9×10^{-9} g of the vitamin increases the rate of CO_2 evolution from 41 mm^3 to 57 mm^3 per hour—or an increase of more than 35% over the original rate. In other words, employing the Warburg technic and propionic-acid bacteria grown in vitamin B_1-deficient media, it is possible to demonstrate the presence of as little as 3.9×10^{-9} g of vitamin B_1. It seems possible to use a bacteriological method for the assay of this vitamin.

The function of the vitamin here is probably not that of co-carboxylase since acetaldehyde has never been detected in a propionic-acid fermentation. More probably it is that of a hydrogen carrier as has already been suggested by Lipman. Whether or not phosphorylation of the vitamin occurs is now being investigated.

The authors are indebted to Dr. H. G. Wood of this laboratory for suggestions and assistance in culturing the propionic-acid bacteria.

10030

Storage of Vitamin D in the Tissues of Growing Calves.*

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Numerous researches have been conducted with the view of determining the influence of varying intakes of vitamin D on the antirachitic potency of milk and eggs. Very few data are available, however, relative to the influence of vitamin D intake on the storage of this vitamin in the body tissues of various types of animals.

Metz and Coppens[1] have reported that some of the parenchymatous tissues from dogs contain considerable quantities of vitamin D. DeVaney and Munsell[2] have reported on the vitamin D content of ox, lamb, swine and calf livers, as purchased on the open market. Heymann[3] investigated the storage of vitamin D in various tissues of the rabbit, as resulting from feeding definite unitage of this vitamin in the form of viosterol in oil. According to this investigator, the amount of vitamin D storage was relatively small in all cases, the greatest storage being in the liver and in the blood, respectively.

It is highly probable that the lack of data concerning the ability of the larger animals to store vitamin D in their tissues is due to the amount of time and expense involved in carrying out any such investigations. Investigations of this type necessarily require an accurate measure of the total vitamin D intake of the particular animal while being maintained under carefully controlled conditions during a reasonable period previous to the time of slaughtering. Fortunately, in the investigation herein reported, an unusul opportunity for carrying out an investigation of this type presented itself when the Department of Dairy Husbandry completed some experiments which had been designed to determine the vitamin D requirement of growing calves.[4] During the above investigations a careful record had been kept of the total vitamin D intake and of the changes in body-weight of young calves from 2 weeks to 7 months of age. When the above animals were slaughtered at the end of the investigation, the tissues for the following studies were obtained.

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* Authorized for publication on May 23, 1938, as paper No. 839 in the Journal Series of the Pennsylvania Agricultural Experiment Station.

[1] Metz, G. A., and Coppens, Ph. A., *Nederland. Tijdschr. v. Geneesk.*, 1934, **78**, 769.

[2] DeVaney, G. M., and Munsell, H. E., *J. Home Ec.*, 1935, **27**, 240.

[3] Heymann, Walter, *J. Biol. Chem.*, 1937, **118**, 371.

Experimental. The calves from which the tissues used in these studies were taken were of the Holstein breed and were approximately 2 weeks of age when placed on experiment. These animals were maintained at all times in individual stalls in a darkened barn. Each calf was fed a diet consisting of a mixture of ground grains and skim milk powder and, in addition, received some roughage either in the form of night harvested dehydrated alfalfa hay or beet pulp. The various ingredients of the diet were so chosen as to formulate a mixture as low as possible in vitamin D but complete in all other known dietary respects. Each ingredient was subjected to biological assay for its vitamin D content previous to being incorporated into the ration.

In addition to this vitamin D-deficient basal diet, each calf, except those retained as negative controls, received carefully measured quantities of vitamin D as a dietary supplement. Some of the calves received the vitamin supplement in the form of a cod liver oil concentrate while other calves received the supplement in the form of irradiated yeast.

The length of time during which vitamin D supplements were fed before the animals were slaughtered varied somewhat with the different animals, ranging from 164 to 210 days. At the end of these periods the various animals were slaughtered in the usual manner and the desired samples taken for assay.

In preparing the samples for assay, the various tissues were taken from the animal body (in whole) as soon as possible, freed of connective tissue, weighed, macerated by passing through a meat chopper, immediately frozen and stored in this condition until ready for use. In the preparation of blood samples, an indefinite volume of the fluid (about 1000 ml) was collected in an oxalated glass container and stored at a low temperature until reduced to a suitable state for the feeding tests.

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[4] *Research Bulletin* 364, Pennsylvania Agr. Exp. Station.

From calves selected from the first series of studies, the liver, heart, brain, kidneys and a part of the blood were retained for biological assay. At first, all of the above tissues except the blood were fed in the original state in sufficiently small daily portions to insure complete consumption. However, it was soon found to be impossible for the test animals to consume sufficient quantities of the fresh tissues to bring about a satisfactory antirachitic response. It was necessary, therefore, to desiccate these tissues in order to overcome this difficulty. Even after dehydrating the tissues, it was still impossible to make an accurate determination of the antirachitic value of brain, heart and kidneys because of the inadequate consumption of these tissues by the test animals. Therefore, from those calves selected from the second series of experiments, only the blood and liver were retained for biological assay.

In order to obtain the vitamin D from the blood and to preserve it in a suitable state for the feeding tests, a fat extraction according to the Rose-Gottlieb procedure was made and the combined ether extracts were reduced to a definite volume, which was proportional to the volume of the original blood used.

All biological assays were carried out according to the U. S. P. technic[5] (1936) of assaying for vitamin D.

The data obtained as the result of determining the antirachitic potency of the bloods and livers from the various calves are given in Table I.

The data presented in the table readily disclose the lack of efficient storage of vitamin D in certain tissues of the calf. In fact, variations in the daily vitamin D intake of as much as twenty-two-fold resulted in only a threefold variation in the antirachitic potency of the bloods and a twofold variation in the antirachitic potency of the livers of the respective calves.

While a definite relationship between the vitamin D intake of the various calves and the amount of this substance found in the blood and in the liver of the respective calves does not appear to exist, the data obtained do indicate that, in most cases, those animals which received the greatest daily intake of vitamin D also stored the greatest amount of the antirachitic substance in their tissues. However, there appeared to be some exceptions to this generalization. In fact, those animals which were selected from the first series of experiments (Calves Nos. 367, 369, 381, 383, and 384) appear to have stored a lower percentage of the vitamin D in their tissues than did animals of the second series (Calves Nos. 409, 410, 411, 416, 419, 421, and 424). A possible explanation for this apparent difference

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[5] *U. S. Pharmacopoeia,* Eleventh Decennial Revision, 1936, p. 482.

TABLE I.

Vitamin D Intakes of Calves During Experimental Periods and Storage of This Vitamin in Blood and Liver.

No. of calf	Duration of feeding test, days	Gain in wt, lb	Supplementary source of Vit. D	Total Vit. D intake, U.S.P. units	Vit. D per 100 ml blood, U.S.P. units	Vit. D per 100 g liver, U.S.P. units	Total Vit. D in blood,* U.S.P. units	Total Vit. D in liver, U.S.P. units
367	164	255	Oil	809,045	13	17	1481	386
369	174	175	”	110,770	7	15	612	265
381	164	255	None	129,695	11	13	1350	282
383	164	254	Yeast	805,810	15	19	1704	444
384	164	148	None	119,865	9	11	805	214
409	182	106	”	39,910	17	—	988	—
410	210	253	Yeast	249,095	21	22	2645	579
411	210	214	Oil	71,005	17	17	1678	361
416	210	200	Yeast	153,785	20	15	1988	328
419	210	166	”	77,495	15	20	1243	449
421	210	190	Oil	130,240	20	22	1750	296
424	203	180	None	48,700	—	17	—	352

*Total volume of blood calculated from the following equation:

$$\text{Vol. of blood in mls.} = \frac{\text{Wt. of calf (in lbs.)} \times 453.6 \times 0.0771}{1.059}$$

in the ability of the 2 groups of calves to store vitamin D in their tissues may be that the 2 groups of calves were born in slightly different seasons of the year. Those calves comprising the first series were born in late November, while those of the second series were born during the first half of September. If this difference in time of birth accounts for the variations in the response of the 2 groups of calves, it is possible that such a variation was due to a combination of the prenatal effect of the antirachitic condition of the cow on its offspring and to differences in the antirachitic potency of the liquid milk which the respective calves received during the first 2 weeks of life. As a whole, those calves comprising the second series received a lower intake of vitamin D during the experimental period than did those of the first series and, in turn, were able to store a larger percentage of this substance.

The 2 sources of vitamin D used in these investigations appear to be equally effective so far as storage of the antirachitic potency in the blood and in the liver of the calf is concerned.

An interesting point to be observed from the above data is the comparatively uniform concentration of vitamin D in the blood and liver of the calf. In fact, in the young of this species it appears that the liver is not an important storage organ for vitamin D. It seems, therefore, that unless there are other tissues of the calf which serve as more efficient storage organs for vitamin D than the liver or the blood, the ability of the calf to store vitamin D is rather limited. These data also indicate that the calf should be relatively susceptible to rickets if it were maintained for any extended period of time on a diet comparatively low in vitamin D and without access to ultraviolet radiation. However, serious outbreaks of rickets among calves maintained on the usual high grade rations have not been reported and in all probability have not been observed because of the appreciable antirachitic content of the sun-cured hays, roughage, and other constituents comprising a diet of this type.

Summary. A study was made of the effect of feeding young calves definite quantities of vitamin D, in the form of cod liver oil concentrate or irradiated yeast for periods of time ranging from 164 to 210 days, on the resultant storage of this substance in the blood and in the liver of the animal. From the data obtained in this study, it is concluded that only a relatively small percent of the antirachitic intake of the calf is stored in the blood and in the liver. The concentration of the antirachitic factor per unit weight was found to be approximately equal in the 2 tissues studied. Natural vitamin D as found in cod liver oil and activated ergosterol as found in irradiated yeast seem to be stored at about the same degree of efficiency.

10031

Action of Oestrin and Progesterone on the Anterior Pituitary.

J. B. Brooksby. (Introduced by J. B. Collip.)

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The reciprocal relationship between the gonads and the anterior pituitary has been the subject of investigation from several angles. Not the least interesting of these is the study of the action of the gonad hormones on the hypophysis, from the point of view of prevention of castration changes and from that of producing cytological changes and macroscopic enlargement in normal and castrate animals. The action of oestrin in this respect has been well established, both as regards the repair of castration changes (Hohlweg and Dohrn,[1] Haterius and Nelson[2]) and production of hypertrophy (Hohlweg,[3] Nelson,[4] Wolfe[5]). The position with regard to the luteal hormone is less clear. Hohlweg and Dohrn[6] report that while 5 to 6 rat units of oestrin daily were necessary to prevent the castration changes in the pituitary of adult female rats, only one rat unit daily was necessary if 0.2 rabbit units luteal hormone were given simultaneously. In 1935, however, Hohlweg, working with crystalline progesterone, reported failure to prevent castration changes in the pituitaries of immature and adult female mice, while Clauberg and Breipohl,[7] using an impure progesterone alone, obtained inhibition of the castration changes in the pituitaries of adult female rats with 7.5 rabbit units daily. While the results of Clauberg and Breipohl might be explained on the grounds of contamination of the sample of luteal hormone with oestrin or androsterone, the earlier experiment of Hohlweg and Dohrn could be explained only on the grounds of a synergism between oestrin and progesterone. The following experiments were made to obtain evidence for or against the synergistic effect and at the same time to obtain data on the effect of progesterone alone.

Two series of experiments were made, differing only in the oestrogen used and the duration of treatment. In each case, 4 groups of 10 3-month-old virgin female rats were taken, and ovariectomized to avoid interference with the results by gonad hormones from the animal's own ovaries. On the day following castration, treatment with solutions in oil of the crystalline hormones was begun. Group A in both series received 500γ progesterone in 0.25 cc of oil daily; group B in the first series, 300γ ketohydroxyoestrin and in the second series, 100γ dihydroxyoestrin in the same volume of oil daily; group C, the same dosage of progesterone and of the oestrin appropriate to the series; while group D remained untreated as controls. After 10 days' treatment in the case of the first series, and 14 days in the second, the rats were killed and the pituitaries removed and weighed. Histological sections of the pituitaries of the animals in series 2 were stained by a modification of Mallory's aniline-blue orange G method and examined for castration changes.

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[1] Hohlweg, W., and Dohrn, M., *Klin. Wchnschr.*, 1932, **11**, 233.

[2] Haterius, H. O., and Nelson, W. O., *J. Exp. Zool.*, 1932, **61**, 175.

[3] Hohlweg, W., *Klin. Wchnschr.*, 1934, **13**, 92.

[4] Nelson, W. O., *Endocrinol.*, 1934, **18**, 33.

[5] Wolfe, J. M., Proc. Soc. Exp. Biol. and Med., 1935, **32**, 757.

[6] Hohlweg, W., and Dohrn, M., *Wien. Arch. inn. Med.*, 1931, **21**, 337.

[7] Clauberg, C., and Breipohl, W., *Klin. Wchnschr.*, 1935, **14**, 119.

The weights of the pituitaries are given in Table I.

TABLE I.
Pituitary Weight (mg).

Castrate Females	Series I Ketohydroxyoestrin		Series II Dihydroxyoestrin		Histology Castration Cells
	Avg	Range	Avg	Range	
Untreated controls	9.8	6-12	9.5	7-12	+
Progesterone-treated	11.4	8.5-14	10.3	8-13	—
Oestrin-treated	14.9	9-26	15.7	10-18	—
Oestrin + progesterone-treated	18.1	11-23	18.7	15-25	—

It can be seen that significant differences exist among the 4 groups. The pituitaries of the untreated castrates do not show the enlargement characteristic of castration which is not usually well developed until after the 14th day from operation, and this factor does not therefore enter into the consideration of the results. The progesterone-treated animals show a slight increase of pituitary weight, which may be significant as the dose of 500γ progesterone is not high in comparison with the dosage of oestrin necessary to produce hypertrophy. The pituitaries of the oestrin-treated animals are markedly increased in weight though the average value of 14.9 mg in Series I is raised by 1 mg on account of the isolated occurrence in the group of a pituitary weighing 26 mg. The remaining values in the group lie between 9 and 17.5 mg, giving an average of 13.8 mg. The weights of the pituitaries of the oestrin plus progesterone-treated group are clearly higher than those of the other groups, presenting good evidence for the existence of the postulated synergism.

The histological findings are summarized in the last column of the table. Well marked castration changes were seen in the untreated controls and similar changes were also noted in the pituitaries of another group, not described, killed 7 days after castration. These changes were absent from the 3 other groups. It appears from this result that progesterone, even in this moderate dosage, has a definite inhibitory effect on the castration changes in the pituitary, and its action is continued over a period in which the departure from normal would have been quite distinct.

The physiological significance of these actions of progesterone is not at present obvious. It may be noted, also, that progesterone is evidently not an essential factor in the production of pituitary hypertrophy. The enlargement can be set up in castrate animals by oestrin alone, and can be continued for a long period in absence of the ovaries, as is shown by the following experiment.

Each of 24 3-month-old female rats was given 100γ of dihydroxyoestrin daily in 0.25 cc oil, the dose being increased to 150γ at the end of 14 days. Vaginal smears were made daily. On the 7th day of treatment, the rats were ovariectomized, when the ovaries were found to have large active corpora lutea. It was noted, confirming the observation of Selye, Collip and Thomson,[8] that the vaginal smears did not become cornified until 5 to 8 days after the operation. Six rats were killed on the 14th, 21st, 28th, and 35th days of treatment. It was found that the pituitary weights continued to increase with the oestrin treatment. The averages of the successive groups killed were: 14.3 mg, 17.4 mg, 23.5 mg, and 26.3 mg. In spite of these findings, the possibility of progesterone being an important factor in the gonad-hypophyseal relationship cannot be neglected, and further work on this problem is in progress.

Summary. It has been shown that 500γ progesterone daily will prevent castration changes in the hypophysis of 3-month-old female rats, and that the same dosage will enhance the action of oestrin in causing macroscopic enlargement of the pituitary.

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[8] Selye, H., Collip, J. B., and Thomson, D. L., PROC. SOC. EXP. BIOL. AND MED., 1935, **32**, 1377.

10032 P

Chemotherapy of Pneumococcal (Type II) Meningitis in the Rat.

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While it has been shown that sulfanilamide produced a high percentage of cures in mice,[1-5] rats,[3, 5-9] and rabbits[10, 11] infected by different routes with pneumococci (Types I, II, III, VII, and XIV), studies of experimental pneumococcal meningitis have not been reported, although successful sulfonamide therapy in human pneumococcal meningitis has been observed.[12, 13, 14]

In this report the results obtained in a preliminary series of 56 rats with Type II pneumococcal meningitis, produced by intracranial inoculation, are presented.

A 25-gauge needle, 3.0 mm long and attached to a tuberculin syringe, was pushed to the hilt through the carbolized skin and the skull of etherized rats at a point about 4 mm to the left of the sagittal suture and half way between eye and ear. The inoculum, consisting of 0.1 cc of an 18-hour broth culture (Binda strain) diluted 10^{-5}, was slowly injected. Two rats, similarly inoculated but with 0.1 cc of the same culture diluted 10^{-6}, died in less than 93 hours.

The progress of the infection was then determined by killing 2 rats 3 and 5 hours after inoculation. Direct smears from the spinal cords (lower lumbar portion) of the 3-hour rats were negative for pneumococci, but contained many polymorphonuclear leukocytes,

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[1] Rosenthal, S. M., *Pub. Health Rep.*, 1937, **52**, 48.

[2] Cooper, F. B., Gross, P., and Mellon, R. R., Proc. Soc. Exp. Biol. and Med., 1937, **36**, 148.

[3] Rosenthal, S. M., Bauer, H., and Branham, S. E., *Pub. Health Rep.*, 1937, **52**, 662.

[4] Schmidt, L. H., Proc. Soc. Exp. Biol. and Med., 1937, **37**, 205.

[5] Cooper, F. B., Gross, P., and Lewis, M., *Am. J. M. Sc.*, in press.

[6] Gross, P., and Cooper, F. B., Proc. Soc. Exp. Biol. and Med., 1937, **36**, 225.

[7] Gross, P., and Cooper, F. B., Proc. Soc. Exp. Biol. and Med., 1937, **36**, 535.

[8] Cooper, F. B., and Gross, P., Proc. Soc. Exp. Biol. and Med., 1937, **36**, 678.

[9] Cooper, F. B., and Gross, P., Proc. Soc. Exp. Biol. and Med., 1937, **36**, 774.

[10] Locke, A., Locke, R. B., Bragdon, R. J., and Mellon, R. R., *Science*, 1937, **86**, 228.

[11] Kreidler, W. A., Proc. Soc. Exp. Biol. and Med., 1937, **37**, 146.

[12] Neal, J. B., and Appelbaum, *Am. J. M. Sc.*, 1937, **195**, 175.

[13] Tixier, L., Eck, M., and Grossiard, *Presse méd.*, 1938, **46**, 599.

[14] Martin, R., *Presse méd.*, 1938, **46**, 599.

whereas the smears from the cords of the 5-hour rats contained in addition many pneumococci. Cultures from all 4 cords and the corresponding heart blood were positive with the exception of the blood of one 3-hour rat. These findings demonstrated that spinal meningitis was well established at the third hour.

Treatments were begun 6 hours following infection. A group of 15 rats were given 100 mg of sulfanilamide* and a second group of 15 were given 100 mg of 4,4′-di-(acetylamino)-diphenylsulfone,† both orally, and the third group of 20 rats were reserved as untreated controls. The 100 mg oral doses were given twice daily for 2 days and then once daily for 8 subsequent days.

The survivors were killed after 3 weeks and cultures and smears made of the heart blood and the distal portion of the lumbar cord from all animals.

All of the 20 control rats died within 68 hours following the inoculation and with an average survival time of 36.5 hours. Of the 15 sulfone-treated rats, 9 (60%) died with an average survival time of 68 hours. In the sulfanilamide-treated group only 4 (26.7%) died and the average survival time of these rats was 8.5 days.

All cultures of both blood and spinal cord of the control animals were positive. There was only one negative cord culture in the fatalities of the sulfone group, while all blood cultures were positive for pneumococci. Among the 4 fatalities of the sulfanilamide group, only one positive cord culture was obtained, although only one animal showed a negative blood culture. All blood and spinal cord cultures of the survivors were negative for pneumococci.

Histologically, the lesions of the rats that died consisted of a thick, fibrino-purulent, pial exudate involving the brain and the entire cord. The ventricles and the central canal of the cord contained pus and extensions of the purulent process into the brain and cord substance through ulcerated foci in the ependymal lining were demonstrable. In some rats there was also superficial cortical and more extensive cerebellar leukocytic infiltration.

Conclusions. A cerebro-spinal meningitis due to a Type II pneumococcus has been produced in the rat. Oral treatment with sulfanilamide, instituted after this disease was well established, resulted in 73.3% cures; with 4,4′-di-(acetylamino)-diphenylsulfone, 40% cures, and with no treatment, 100% fatality. It is quite evident that the sulfone compound was not as effective against this pneumococcal strain as sulfanilamide.

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* Kindly supplied by E. R. Squibb & Sons, New York.

† Synthesized and donated to us by the Monsanto Chemical Company, St. Louis, Missouri.

10033

Studies on the Nutritional Requirements of Hemolytic Streptococci. I. Effect of Various Substances Isolated from Liver Extract on Hemolytic Streptococci.

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Attempts to substitute a relatively simple, chemically defined medium for the usual complex media in the cultivation of hemolytic streptococci have indicated the need for essential accessory growth factors. Considered in themselves multiple or of complex nature, they have hitherto been unidentified. Our preliminary experiments have demonstrated the presence of these indispensable factors in liver extract. A medium of gelatin hydrolysate, amino acids, inorganic salts, and glucose is incapable of inducing the growth of hemolytic streptococci, but the addition of liver extract yields a growth equivalent to that obtained in meat infusion broth. By fractionation glutathione, thiochrome, nicotinic acid, betaine, flavin and glucosamine were isolated from liver extract.† Two to 10 μg of glutathione per cc of medium, 0.00001-0.0001 μg of thiochrome per cc of medium, 40-200 μg of nicotinic acid per cc of medium, 4-20 μg of betaine per cc of medium, 0.01-0.1 μg of flavin per cc of medium definitely contributed to the growth of the Dochez NY5 strain of hemolytic streptococcus while 200 μg of glucosamine per cc of medium noticeably shortened the lag period.‡ These substances are, however, active only with an additional fraction of liver extract of which the complete identity is at this time still undetermined.

Basal Medium:

Gelatin hydrolysate[1]	25	cc
l-Tyrosine	0.1	g
l-Cystine	0.025	”
Distilled water	1000	cc

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* The work of one of us (Y.S.) is financed by the Proctor Fund of the Harvard Medical School and the Milton Fund of Harvard University.

† The details of the isolation and the identification of these substances will be reported in a subsequent paper (Y.S.).

‡ To eliminate the question of adsorption, synthetic products were substituted wherever possible. Synthetic glutathione was kindly supplied by Dr. A. Baird Hastings of the Harvard Medical School. Pfanstiehl's glucosamine was used.

[1] For preparation see Pappenheimer, A. M., Jr., and Johnson, S. J., *Brit. J. Exp. Path.*, 1937, **28**, 239.

Combined, pH adjusted with 5 N NaOH to 7.6, added:		
Calcium alcoholic precipitate of liver extract[2]	0.5	g
Sodium citrate	1.0	"
$NaH_2PO_4 \cdot H_2O$	2.0	"
Mixture heated gently for 10 minutes, filtered. Added:		
d-Glutamic acid	0.2	"
l-Tryptophane	0.1	"
dl-Methionine	0.05	"
dl-Valine	0.05	"
NaCl	1.0	"
$Na_2HPO_4 \cdot 12H_2O$	1.0	"
KH_2PO_4	1.0	"
K_2HPO_4	1.0	"
$MgCl_2 \cdot 6H_2O$	0.3	"

Tubed in 9.0 cc amounts. Volume brought to 9.7 cc with the addition of test substances and distilled water. Autoclaved 10 min. at 112°C. 0.1 cc flavin previously autoclaved in acid solution (pH 4.5) for 10 minutes at 112°C, 0.1 cc 50% glucose solution also autoclaved separately, and 0.1 cc culture added to test media. Final volume 10 cc.

Note: The amino acids and inorganic salts were included on general grounds. Detailed studies on the amino acid and inorganic salt requirements for growth have been deferred until all accessory growth factors have been determined.

Inoculum: A culture of the Dochez NY5 strain of hemolytic streptococcus grown for 6 hours in meat infusion broth at 37°C was centrifuged, washed thoroughly in sterile distilled water, and resuspended in sterile distilled water. It was then inoculated in 0.1 cc amounts.

Incubation: 40 hours at 37°C.

Readings: For greater accuracy direct readings based on turbidity were made by means of a modified Gates nephelometer,[3] rather than by visual approximation. A 2.3 reading was obtained for the growth of the Dochez NY5 strain in meat infusion broth§ incubated for 40 hours at 37°C. Readings of the growth in different test media varied from 2.9 to more than 5.0 (limit of direct reading).

Results: A summary of typical results is presented in Table I.

Conclusion. Glutathione, thiochrome, flavin, nicotinic acid, betaine and glucosamine, in the presence of a calcium-alcoholic precipitate of a highly purified liver extract,** are clearly significant for the growth of the Dochez NY5 strain of hemolytic streptococcus in a deficient medium. The presence of all these factors provides almost optimum conditions: omission of one or more decreases the amount of growth.

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[2] Subbarow, Y., Jacobson, B. M., and Fiske, C. H., *New Eng. J. Med.*, 1936, **214**, 194.

[3] Feemster, R. F., Wetterlow, L. H., and Cianciarulo, J., *Am. J. Public Health*, 1936, **26**, 1176.

§ A beef-heart infusion containing 2% peptone, 0.5% NaCl, and 0.1% dextrose.

** For the supply of liver extract we are indebted to Dr. Guy W. Clark of the Lederle Laboratories, Pearl River, N. Y.

TABLE I.

Influence of Accessory Substances on Growth of Dochez NY5 Strain of Hemolytic Streptococcus.
Control: Growth in Meat Infusion Broth. Nephelometer Reading = 2.3.

Basal Medium containing Ca-alc. ppt. of liver extract + 200 μg of glucosamine per cc of medium	Micrograms per cc. medium	Nephelometer Reading 40 hr	Micrograms per cc Medium	Nephelometer Reading 40 hr	Micrograms per cc Medium	Nephelometer Reading 40 hr
Basal Medium	—	>5.0*	—	>5.0	—	>5.0
Added individually to Basal Medium:						
Glutathione (G)‡	10	4.9	2	> "	0.4	> "
Flavin (F)	0.1	>5.0	0.1	> "	0.1	> "
Nicotinic Acid (N.A.)§	200	> "	200	> "	200	> "
Thiochrome (T)	0.00001	> "	0.00001	> "	0.00001	> "
Betaine (B)§	4	> "	4	> "	4	> "
Added in combination to Basal Medium:						
(G) + (N.A.)	as above	4.9	as above	> "	as above	> "
(G) + (T)	"	"	"	> "	"	> "
(G) + (B)	"	"	"	> "	"	> "
(G) + (F)	"	4.5	"	> "	"	> "
(G) + (T) + (B)	"	4.9	"	> "	"	> "
(G) + (N.A.) + (T)	"	"	"	> "	"	> "
(G) + (N.A.) + (B)	"	"	"	> "	"	> "
(G) + (F) + (B)	"	4.5	"	> "	"	> "
(G) + (F) + (T)	"	4.3	"	> "	"	> "
(G) + (F) + (N.A.)	"	3.9	"	4.7	"	> "
(G) + (N.A.) + (T) + (B)	"	4.9	"	>5.0	"	> "
(G) + (F) + (T) + (B)	"	4.3	"	> "	"	> "
(G) + (F) + (N.A.) + (T)	"	3.5	"	4.5	"	> "
(G) + (F) + (N.A.) + (B)	"	3.3	"	4.0	"	4.8
(G) + (F) + (N.A.) + (T) + (B)	"	2.9†	"	3.6	"	4.7

*Nephelometer Readings of 5.0 or more indicate essentially no growth.

†Nephelometer Readings of 3.0 or less indicate good growth.

‡No growth without glutathione.

§Although these substances as such were isolated, we have reasons to believe that there are still others in liver extract closely allied but more active.

10034 P

Excretion of Insulin by Normal and Pathologic Rabbit Kidney.

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Recently, Jourdonais and one of us[1] demonstrated the presence of insulin in the blood stream for 60 to 90 minutes after the intravenous injection of massive doses of insulin in rabbits. After that period, insulin was not detected in the blood by the procedures used. To determine the fate of at least part of the injected insulin, the present experiments on the renal excretion of this hormone were planned. Moreover, since insulin is readily adsorbed to proteins, we endeavored to determine whether insulin is lost in greater amounts through the pathologic kidney exhibiting proteinuria than through the normal kidney. These preliminary studies will indicate our results to date.

Three rabbits received 200 units of insulin* intravenously and one the same amount subcutaneously. Urine specimens (by catheter) were collected before and at regular intervals after the injection of insulin for the next 5 or 6 hours. The bladder was washed with 2 or 3 cc of water after the collection of each urine specimen. Gentle abdominal massage over the bladder area was then employed to insure complete emptying of the bladder. All urines, including the controls, were preserved by the addition of 2 or 3 drops of 1/1000 solution of merthiolate (Lilly). Thirty urine specimens, including 6 controls, were obtained from these 4 rabbits, each of which was injected intravenously into a fasting test rabbit, in most instances within 48 hours of its collection.

Proteinuria was induced in 3 rabbits by the subcutaneous injection of 2 mg per kg of uranium nitrate. The same experimental procedure as outlined above was carried out on the pathologic rabbits. Eighteen urines, including 3 controls, were obtained from these animals; these were injected intravenously into fasting test rabbits.

Table I represents one of the 4 experiments carried out on the excretion of insulin by the normal rabbit kidney, and Table II one

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[1] Jourdonais, L. F., and Bruger, M., unpublished results.

* 10 mg of insulin powder assaying about 20 units per mg dissolved in 5 cc of physiological saline. We are indebted to the Eli Lilly Company for a generous supply of this insulin.

TABLE I.

Adult White Rabbit, Male, Fasting 24 Hours, Urethral Catheterization. 250 cc Water by Stomach Tube Evening Before Experiment.

Time	Specimen	Vol. of urine cc	Blood Sugar of expt rabbit mg%	Blood sugar of test rabbits after urine injection				Max. decrease of blood sugar after urine injection mg	Remarks
				Control mg%	½ hr mg%	1 hr mg%	2 hr mg%		
9:30-10:00	Control urine 1	7.6		101	93	102	97	8	
10:00-10:30	” ” 2	6.6		118	123	125	111	7	
11:00	Control blood		150						200 units of insulin intravenously at 11:05
11:00-11:30	Expt urine 1	7.0		115	57	37	47	78	Convulsions in test rabbit
11:20	Blood (15 min after insulin)		167						
11:35	” (30 min)		152						
11:30-12:00	Expt urine 2	5.7		94	74	79	60	34	Trace of blood in urine
12:05	Blood (1 hr)		81						
12:35	” (1½ hr)		82						
1:05	” (2 hr)		67						
1:00-1:30	Expt urine 3	6.4		104	75	71	75	33	+++ to ++++ blood in urine in this and in subsequent specimens
2:05	Blood (3 hr)		63						
2:00-2:30	Expt urine 4	9.2		96	80	96	103	16	
3:05	Blood (4 hr)		47						
3:00-3:30	Expt urine 5	4.8		96	112	100	116	0	
4:05	Blood (5 hr)		52						
4:00-4:30	Expt urine 6	5.6		115	109	111	127	6	
5:05	Blood (6 hr)		44						Rabbit died at 6:15 p.m.

TABLE II.

Adult White Rabbit, Male, 2.5 kg Weight, 5 mg Uranium Nitrate Subcutaneously 48 hr Before Experiment. 200 cc Water by Stomach Tube 30 min Before Experiment.

Time	Specimen	Vol. of urine cc	Blood Sugar of expt rabbit mg%	Blood sugar of test rabbits after urine injection				Max. decrease of blood sugar after urine injection mg	Remarks
				Control mg%	½ hr mg%	1 hr mg%	2 hr mg%		
11:15	Control urine	3.0		100	103	114	120	0	Contains 0.436 g of protein per 100 cc of urine
11:15	” blood		140						200 units of insulin intravenously at 11:20
11:20-11:50	Expt urine 1	5.5		107	50	29	55	78	Convulsions in test rabbit
11:50	Blood (30 min after insulin)		165						
11:50-12:20	Expt urine 2	4.7		103	64	44	64	59	Blood macroscopic in all urines except control
12:20	Blood (1 hr)		119						
12:20-1:20	Expt urine 3	5.0		99	58	54	57	45	
1:20	Blood (2 hr)		77						
1:20-2:20	Expt urine 4	4.0		94	110	104	120	0	
2:20	Blood (3 hr)		61						
2:20-3:30	Expt urine 5	7.0		104	114	116	125	0	
3:20	Blood (4 hr)		64						Rabbit found dead in cage the following morning

of the 3 experiments on the excretion of insulin by the rabbit kidney rendered pathologic with uranium nitrate. These protocols show (1) insulin is not a constituent of the urine of normal rabbits or of the urine excreted by the rabbit kidney rendered pathologic with uranium nitrate, at least in the quantities employed. (2) Following the intravenous administration of insulin, insulin or an insulin-like substance† is excreted readily by the rabbit kidney. The greatest concentration of insulin in the urine occurs directly after its administration, the rate of excretion tapering off until 3 to 3½ hours later when insulin no longer could be detected in the urine. (3) After the intravenous administration of insulin, this hormone is not excreted in greater amounts by the pathologic kidney than by the normal rabbit kidney.‡

10035

Influence of Thyroid Hormone on Estrin Action.

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The interference of hyperthyroidism with the response of castrate female rats to estrogenic hormones was first reported by Reiss and Perény.[1] These authors came to the conclusion that there is an antagonism between the two hormones. Their observation has been confirmed later by Weichert and Boyd[2] and Van Horn.[3] The latter advanced the explanation that the increased metabolism obtained by thyroid feeding brings about a more rapid elimination of the estrogenic substance.

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† Although no definite proof can be offered to substantiate the claim that this insulin-like substance is insulin, the following will be of interest. 148 cc of urine was obtained from 1 rabbit within a period of 4 hours after the intravenous injection of 200 units of insulin. A modified Dudley's procedure (picric acid precipitation) was then employed for the precipitation of insulin from this urine. The final precipitate thus obtained, though very small in quantity, was dissolved in distilled water and the solution injected subcutaneously into a fasting rabbit. In 2 hours, the blood sugar decreased from 132 mg to 66 mg %. Evidently, this hypoglycemic principle present in rabbit urine after the administration of insulin, either is insulin or a substance having identical properties.

‡ Since blood was present frequently in the urines collected after the injection of insulin (due to trauma or to a nephrotoxic action of the insulin protein), it is possible that the insulin detected in the urine was not eliminated by the kidney but represents contamination with blood containing insulin. For this reason, the amount of blood present in the urine was quantitated chemically during those times when this blood was known to contain insulin; values of 1 part of blood in 2000 parts of urine to 1 in 5000 were obtained. Obviously, the quantity of blood present in the urine could not have accounted for its content of insulin.

[1] Reiss, M., and Perény, S., *Endokrinologie*, 1928, **2**, 181.

In all these experiments, a rather large dose of thyroid has been employed over a relatively long period, producing a considerable and sustained increase of metabolism. The present investigation was suggested by the idea that quantities of thyroid, too small to produce an appreciable increase of metabolism, might have a reversed action on the estrin effect, sensitizing the animal to estrogens. This expectation was admissible in view of the fact that thyroid at low dosage exerts an anabolic effect in contrast to the catabolic action at medication with larger quantities. This latter contention is based on the clinical observation that emaciated patients frequently gain weight when small doses of thyroid are given. Our own observation in thyroidectomized rats is also in agreement with this view. The repair of loss in weight suffered through the operation is extremely slow for a long time, but rapid gain follows medication with minute doses of thyroid. Whether or not the non-thyroxine fraction of the thyroid gland might act differently than the thyroxine fraction in this respect, was a second question to be investigated.

Female rats 80 days of age and of established estrous cycle were castrated and after allowance of a reasonable time for recovery tested for absence of estrus. For priming, a safe excess of urinary estrin was used and thereafter the minimum effective dose was determined. The total quantity of estrin given to induce estrus in a group of rats was termed a unit. Injections were given 6 times, 3 each on 2 consecutive days. Vaginal smears were taken repeatedly on the fourth and fifth day. Only perfect estrus was counted as positive response; proestrus reaction was not counted. Rats not showing full estrus after treatment with the dose that produced the effect in the majority of animals of the group (with 1 unit), were discarded. With consideration of the possibility that treatment with thyroid might have an indefinite influence over a prolonged time and complicate subsequent medication, each animal was used only for a single experiment.

The thyroid preparations were given by mouth; in the case of thyroxine, a stomach tube was used, in order to avoid any possible loss of substance. Perfect ingestion of the accurately determined quantity was secured in every instance.

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[2] Weichert, C. K., and Boyd, R. W., *Anat. Rec.*, 1933, **58**, 55.

[3] Van Horn, W. M., *Endocrinology*, 1933, **17**, 152.

The following preparations were used in these experiments:

1. Crystalline racemic thyroxine.

2. Dry thyroid substance of 0.46% iodine content, *i. e.*, more than twice U.S.P. strength.

3. Thyroid globulin, prepared by extraction of fresh pig's thyroid; it contained 0.565% iodine, 0.14% of which was in form of thyroxine iodine as determined by the method of Leland and Foster.[4] In consideration of a probable error of 15%, as calculated by these authors, the figure would have to be increased to about 0.165%, corresponding to 0.25% thyroxine.

4. An acid soluble fraction, prepared by alkaline hydrolysis of the previous preparation. This preparation had an iodine content of 0.26%; although supposed to contain only the diiodotyrosine fraction, its chemical assay still showed 0.0085% of thyroxine iodine or 0.01% corrected.

The approximate calorigenic effect of the thyroid preparations, as determined in preliminary metabolic experiments, was estimated to be at least 3 times that of the corresponding thyroxine content. The metabolic effect in castrate females, however, was not determined. The experiments performed are compiled in Tables I-III.

The results seem to permit the following conclusions:

Table I. Treatment with thyroid material at moderate but efficacious doses for 3 days does not interfere markedly with the action

TABLE I.

Single Dose		Times	Days of medication	Units Estrin	Days of injection	Reaction		Remarks
						pos.	neg.	
1. Thyroxine	.5 mg	1	1	1	3 and 4	6	0	
2. ”	.33 ”	3	1-3	1	5 and 6	6	0	continued
Thyroid*	30 ”	10	11-20	1	19 and 20	0	8	test
3. Thyroxine	.6 ”	3	1-3	1.5	5 and 6	6	1	
4. Thyroglobulin†	30 ”	5	1-5	1.4	7 and 8	4	2	continued
”	30 ”	11	11-22	1.4	21 and 22	0	6	test

*This dose is equivalent in metabolic effect to 0.2 mg thyroxine, by estimation.
†Equivalent to 0.225 thyroxine.

TABLE II.

Single Dose		Times	Days of medication	Units Estrin	Days of injection	Reaction		Remarks
						pos.	neg.	
Thyroglobulin	4 mg	5	1-5	0.8	7 and 8		8	continued
”	4 mg	11	11-22	0.8	21 and 22		8	test

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[4] Leland, J. P., and Foster, G. L., *J. Biol. Chem.*, 1932, **95**, 165.

TABLE III.
Acid soluble thyroid fraction, 100 mg, equivalent in activity to approximately 0.12 thyroxine.

Single Dose	Times	Days of medication	Units Estrin	Days of injection	Reaction		Remarks
					pos.	neg.	
1. 250 mg	1	1	0.6	2 and 3		9	continued
200 "	3	10-12	0.6	14 and 15		9	test
200 "	11	21-32	1.0	29 and 30	2	7	
			1.0	39 and 40		9	
			1.50	49 and 50	9		
2. 250 "	8	1-6, 8, 9	1	8 and 9	7	1	
After 3 weeks	without	medication	1		1	7	
" 4 "	"	"	1.2		6	2	
100 "	5	1-5	1	7 and 8	5	2	continued
100 "	11	11-22	1	21 and 22		7	test
100 "	5	1-5	1.5	7 and 8	7		continued
100 "	11	11-22	1.5	21 and 22	7		test

of estrin; treatment for more than 5 days increases the threshold for estrin.

Table II. Thyroid globulin at subminimal doses does not sensitize the animals to the action of estrin.

Table III. A product low in thyroxine, but high in the diiodotyrosine fraction, given at a comparatively low, but on prolonged administration efficacious, dosage does not sensitize to estrin during a few days of medication. A calorigenic effect cannot yet be supposed after this short treatment. Longer treatment causes interference. This effect continues increasing for some time after cessation of treatment. At the doses used, the estrin requirement for induction of estrus is not increased beyond 50%.

The impression is gained that the need for increased estrin after thyroid treatment is dependent on the increase in metabolism, be it that estrin is metabolized at a proportionately augmented rate or that the tissues in stimulated activity require larger quantities of estrin to respond.

10036 P

Metabolism in Normal and Thyroidectomized Rats as Influenced by Thyroxine and Thyroid Globulin Feeding.

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The evaluation of thyroid preparations is still in an inchoate state, because (1) the accepted method, based on iodine content, does not give an estimation of the physiologic activity, since the thyroxine component of standard products may vary considerably (up to 600% as found by Harrington and Randall[1]); (2) the methods of thyroxine assay available give more comparative than absolute values; (3) the physiologic action of thyroid substance or extract seems to depend partly on the chemical linkage between the thyroxine and other groups present. While the investigation of Thompson and co-workers[2] showed that the potency of thyroid substance is considerably reduced by hydrolysis, which would break up such linkages, Palmer *et al.*[3] concluded from their experiments on guinea pigs that the calorigenic action of thyroid substance is a direct function of the thyroxine contained, which, however, is present in the *l*-form and twice as potent as the commonly prepared racemic mixture.

In our attempt to find a reliable and fairly accurate method of bioassay, we used first normal and later thyroidectomized male rats, finding in the latter a far more suitable test animal. In this we are at variance with the work of Gaddum,[4] which is probably explainable by the difference in the age of the rats used.

A closed circuit apparatus was used, as described by Benedict.[5] The normal rats used were 80-100 days of age. Thyroidectomy was performed when the rats were 70-80 days old. In the latter case, at least 3 weeks were allowed for recovery. The rats were starved for 20 hours and allowed to remain for 30 minutes in the respiration chamber before testing. The temperature was kept constant at 30° throughout the experiments. The time necessary for the consumption of a constant volume of oxygen was measured, and results calculated as mg O_2 per hour per kg weight. Readings were accepted only when the animal remained quiet but not asleep, and at least 2 series had to agree on the same day. Two consecutive tests several days apart had to agree before medication was given. Such rats that did not remain quiet enough to give uniform results or showed abnormalities (*e. g.* tetany) after operation were eliminated. The percentage of normal as well as thyroidectomized rats that had to be rejected in proper selection of animals was quite considerable. Each animal was used only once for a medication, to avoid the possible influence of progressing age. As standard medication we used crystalline *d,l*-thyroxine, dissolved in slightly alkalinized water. In comparison, a thyroid globulin preparation was used, containing 0.565% total iodine, 0.14% of which was present as thyroxine iodine, determined by the method of Leland and Foster.[6] Considering an estimated loss of 15% in the determination, the corrected value is 0.165%, equivalent to a content of 0.25% thyroxine. Both preparations were fed by stomach tube in accurate quantities in proportion to body weight on 3 consecutive days. Metabolism was determined on the fifth day, at which time the peak of the metabolic curve is reached. Preliminary tests leading to this technic will be reported later in a complete publication.

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[1] Harrington, C. R., and Randall, S. S., *Quart. J. Pharm.*, 1929, **2**, 501.

[2] Thompson, W. O., Thompson, P. K., Taylor, S. G., III, and Dickie, L. F., *J. Clin. Invest.*, 1937, **16**, 479.

[3] Palmer, W. W., and Leland, J. P., *J. Clin. Invest.*, 1935, **14**, 619; Foster, G. L., Palmer, W. W., and Leland, J. P., *J. Biol. Chem.*, 1936, **115**, 467.

[4] Gaddum, J. H., *J. Physiol.*, 1930, **68**, 383.

[5] Benedict, F. G., *J. Nutrition*, 1930, **3**, 161.

TABLE I.
Normal Rats Fed Thyroxine.

No. of rats	1	1	5	1	3
Dose, micrograms	31	28	25	15	10
% increase of metabolism	39	47	20-41	18	0-29

TABLE II.
Thyroidectomized Rats Fed Thyroxine.

No. of rats	1	1	1	1	3	2	2	6	6
Dose, micrograms	25	12.5	10	6	3	2	1	0.75	0.5
% increase in metabolism	46	46	45	42.5	35-39	27-36	27-35	25-36	5-12
Avg	46	46	45	42.5	37	32	31	28.8	9.6

TABLE III.
Thyroidectomized Rats Fed Thyroid Globulin.

No. of rats	5	6	9	6
Doses of thyroglobulin	100	75	50	40
Equivalent to thyroxine	0.25	0.19	0.125	0.1
% increase in metabolism	28-36	18-31	13-20	3-10
Avg increase	31	24	16	7

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[6] Leland, J. P., and Foster, G. L., *J. Biol. Chem.*, 1932, **95**, 165.

Results: The normal rats used had an oxygen consumption varying from 1420 to 1670 mg O_2 per kg per hour, but for the individual rat the figure was constant within a limit of 8%. The O_2 consumption of thyroidectomized rats was 900 to 1200 mg per hour per kg. Medication with varying doses of thyroxine in micrograms per 10 g weight and given daily for 3 days, and corresponding medication with thyroid globulin, calculated on thyroxine content, gave the results presented in Tables I-III.

While it seems that in normal rats the dose of 25 γ thyroxine might fall within the range of best measurable response, the spread of figures is too wide for acceptable conclusions to be drawn. Thyroidectomized rats are about 30 times more sensitive. Although the number of experiments presented so far is limited, a tentative interpretation of results seems to be permissible. They indicate that the percentage metabolic increase plotted as a function of dosage follows a logarithmic curve. A minimum dose is required before any response obtains. Increase of dosage produces initially a rapid increment of metabolism, which approaches a more gradual linear function at 20 to 35% increase and finally becomes slower. For comparative measurements, an increase between 25 and 30% seems to be the most favorable range. Surmising that thyroxine is the sole stimulator of metabolism in the thyroid globulin, between 0.19 and 0.25 γ thyroxine given in this form appears to be as active as 0.75 γ crystalline thyroxine, which would mean that thyroxine in natural linkage is 3 to 4 times more potent than the racemic substance.

10037

Hemolysin in the Urine in Aplastic Anemia.

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Ample evidence is at hand to prove that a substance which is lytic for erythrocytes is present in the urine of human beings under normal conditions. Few studies are available of this phenomenon in patients with disorders of the blood in which hemolysis might be a factor. Accordingly, the urines of patients with certain representative types of blood dyscrasia have been investigated for their content of lysin. In the urine of patients with aplastic anemia lysin has been found to be present in a form which is different from that described for any normal or any pathologic state studied hitherto.

McKee[1] first observed the lytic activity of human urine. Ponder[2] found lysin in more than 90% of normal human urines and states that although it may be absent on any one day it appears regularly in all normal individuals. Abels[3] in a detailed study of the subject examined 2,026 normal urines and found lysin in 93%. No individual secreted non-lytic urine for more than 4 days in succession. The urines from 72 individuals were examined for periods of 2 to 5 weeks and those of 400 individuals for 1 day only. The lysin was heat stable, alcohol soluble, diffusible, adsorbable, destroyed finally by alkali, and inhibited by plasma proteins, lecithin and cholesterol.

For the simple determination of lytic effect, human erythrocytes were washed 3 times in 5 volumes of isotonic saline and made up in a concentration of 1% in saline. Urine samples measuring 0.5, 0.25 and 0.125 cc were measured and mixed with 1 cc of the suspension of erythrocytes. The tubes were kept at 37° in a bacteriological incubator for 4 hours. The titre of the urine lysin is indicated by the lowest dilution of urine which effects complete lysis of erythrocytes in that time.

For the recovery of lysin from the non-lytic urine of cases of aplastic anemia, 1 volume of urine was brought to pH 1 with 6 N HCl. This was refluxed for 30 minutes, cooled, brought to pH 6 with 2 N NaOH and evaporated to dryness on the water bath. The residue was ground with 1 volume of 95% alcohol for 15 minutes and filtered. The precipitate was washed twice with one-fourth volume of 95% alcohol with repeated grinding and the washings were added to the filtrate. The whole was evaporated to dryness on a boiling water bath. The residue was taken up in one-half volume of isotonic saline filtered and adjusted to a pH of 5.6. Of this final saline solution 0.5 cc was added to 1 cc of 1% suspension of washed human erythrocytes and placed in an incubator at a temperature of 37°C. The tubes were examined, without removing them from the incubator, every 5 minutes for 15 minutes and at intervals of 10 minutes thereafter.

All of the 8 cases of aplastic anemia tested were typical. In 5 the diagnosis had been confirmed by examination of the bone marrow. In all cases repeated transfusions of blood as well as heavy treatment with liver extract and with iron failed to cause improvement. All these patients had been ill with anemia for months or years, and showed little or no evidence of regeneration in the peripheral blood.

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[1] McKee, C. S., *Brit. Med. J.*, 1915, **2**, 596.

[2] Ponder, E., *Brit. J. Exp. Path.*, 1921, **2**, 34, 192.

[3] Abels, J. C., *Am. J. Physiol.*, 1934, **107**, 603.

Table I shows the results of testing normal urine for the presence of lysin. Of 2,460 tests, 2,234 were lytic in the experiments of Ponder and of Abels. In Table II are shown the results of testing 89 specimens from 8 patients with aplastic anemia. In no instance was any lytic activity present on direct test even though the observations were continued over weeks. It has been previously noted that normal urines of pH higher than 6.2 were almost invariably non-lytic unless extremely ammoniacal. In the present study of 89 non-lytic specimens, only 4 were of a pH greater than 6.2. Thus this finding was completely contrary to any result obtained in normals and warranted further study. We felt, therefore, that if the lysin were excreted it must be in some bound or conjugated form; and that if mildly hydrolyzed at pH 1 it would again be demonstrable. We had previousy noted[3] that the lysin in the urine could withstand such acid treatment with unimpaired activity.

TABLE I.

Controls from average population	Period of observation	Lytic specimens No.	Lytic specimens %
2,460	1 day to 4 months	2,234	91

TABLE II.
Hemolysin Before Hydrolysis in Urine of Patients with Aplastic Anemia.

Patients	No. of specimens	No. of days	No. lytic
M.J.	22	18	0
A.C.	21	17	0
D.L.	19	16	0
G.M.	14	12	0
M.R.	7	7	0
C.D.	5	5	0
L.C.	2	2	0
C.J.	2	2	0
Total	92		0

Accordingly, the non-lytic urines were refluxed for 1 hour at 100°C at pH 1, neutralized to 5.6, evaporated to dryness, and the residue extracted with alcohol, since it was known that the lysin of the normal urine is soluble in alcohol. As controls, 4 normal urines which were known to be lytic were subjected to the same procedure and also 4 normal urines which had been found to be non-lytic. The results are shown in Table III. Of the urines of patients with

TABLE III.
Hemolysin After Hydrolysis in Urine of Patients with Aplastic Anemia.

Patients	No. of specimens	No. of days	No. lytic	Time required for lysis
				min
J.J.	8	8	6	10-10-15-30-30-30
A.C.	4	4	3	5-15-15
D.L.	8	8	8	5-5-20-30-30-40-40-40
G.M.	4	4	4	5-20-20-20
M.R.	5	5	4	10-15-20-45
C.D.	5	5	5	15-20-25-40-40
L.C.	2	2	2	25-35
C.S.	2	2	2	15-15
Total	38		36—94%	
Normals				
J.A. (lytic)	4	4	4	
J.A. (non-lytic)	4	4	0	

aplastic anemia that were tested, none were lytic before hydrolysis, but after hydrolysis 88% were productive of lysis. In the control tests, on the other hand, no lysin was present after hydrolysis in the normally non-lytic urines, though it was still present after hydrolysis in the normally lytic urines.

As further controls, urines were examined from patients with pernicious anemia, Hodgkin's disease, and microcytic anemia due to lack of iron. The results are shown in Table IV. Over a period of 54 days, 62 urines were examined, and of these 55 showed lysis—approximately the expected result in normals.

TABLE IV.
Hemolysin in Urine of Patients with Blood Dyscrasias Other Than Aplastic Anemia.

Cases	No. of specimens	No. of days	No. lytic
Pernicious anemia	16	14	14
” ”	20	16	19
Microcytic ”	16	14	14
Hodgkin's disease	10	10	8

From the results it is clear that a lysin which is present in the untreated urine of normal individuals, of patients with pernicious anemia, and of patients with microcytic anemia, is not demonstrable in the untreated urine of patients with aplastic anemia. By mild acid hydrolysis the lytic property may be restored in the urine of patients with aplastic anemia. This is an abnormal finding, however, since no lysis is caused by normally non-lytic urine from normals or from patients with other disease states after acid hydrolysis. Suitable controls establish the fact that the lysis is not due to pH, to tonicity, or to the ordinary physical factors productive of breakdown of erythrocytes. It seems probable that the normal lysin is present in the urine of aplastic anemia but is excreted in a bound form. Studies of Ponder[4] and of Ponder and Abels[5] suggest that the action of the ordinary inhibitors of lysis is a physical one on the erythrocyte and not a combination of inhibitor with lysin which renders the lysin inert.

Because of the relatively weak hydrolysis required to free lysin from the bound form in urines from patients with aplastic anemia, it is reasonable to suspect that some loose linkage, such as a glucuronate, is present rather than some more permanent conjugation.

Further studies of the nature of the lysin and of the method of conjugation are in progress.

Summary and Conclusions. 1. The urine of patients with aplastic anemia contains no lysin for erythrocytes when tested directly. 2. Lysin appears after hydrolysis at pH 1 and 100°C for 1 hour. 3. Such hydrolysis does not result in the production of lysin in urines which are non-lytic normally.

10038 P

The Comb of the Baby Chick as a Test for the Male Sex Hormone.

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This work is a continuation of that already reported[1] on the use of comb growth in white leghorn chicks for the assay of male sex hormone activity. The method previously employed involved the daily application of 0.1 cc of sesame oil, containing crystalline androsterone, to the region of the comb; the chicks were started on the 6th day after birth, treatment was continued for 10 days, and the combs were excised and weighed on the 17th day; the total dosage varied from 0 to 500 gamma, which corresponds to 0-50 gamma per daily application. In all of these experiments, there occurred a progressive increase in comb weight with dosage of androsterone, but the graphs were highly irregular and curved toward the dosage axis as if they might reach plateau values beyond 500 gamma. The chief aim of the present work was to determine what changes in the experimental conditions previously employed must be made in order to effect: (1) An increase in *uniformity* of the comb growth response of any one group of chicks treated simultaneously with the same quantity of hormone, this uniformity being measured by the standard deviation of the mean for the entire group. (2) An increase in *sensitivity* of the reaction, as measured by the mean difference in comb weight per unit weight of androsterone throughout the entire range of dosage employed.

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[4] Ponder, E., *Proc. Roy. Soc.*, 1925, **98**, 484.

[5] Ponder, E., and Abels, J. C., in press.

*Hiram N. Vineberg Research Fellow in Gynecology.

[1] Frank, R. T., and Klempner, E., PROC. SOC. EXP. BIOL. AND MED., 1937, **36**, 763.

To attain this objective, it has been found desirable to introduce the changes in procedure indicated in Table I.

TABLE I.

Experimental conditions	Previous study	Present study
Age of chick at beginning of application (days)	6	3
Duration of application (days)	10	7
Frequency of application	Daily	Daily
Range of total weight of androsterone per chick (gamma)	0-500	0-50
Volume of sesame oil used daily (cc)	0.1	0.05
Precautions concerning droppings, contact of chicks, segregation of different groups, uniformity of lighting conditions	Maintained throughout	

The use of younger chicks and the reduction of the period of application of the androsterone resulted in a marked increase in *uniformity* of the data; an improvement likewise resulted from the reduction in volume of oil used as vehicle, presumably because of diminished loss by spreading beyond the comb area. The *sensitivity* of the reaction was increased considerably by reducing the dosage range as indicated.

In all, 5 independent series of experiments have been performed, the number of chicks in any one series varying from 70 to 204. With one exception, each series was made up of 6 groups of chicks, each group corresponding to a different dosage value from 0 to 50 gamma inclusive. The final data were plotted as mean comb weight for all the chicks of any one group against the dosage for that group. The graph for any one series approximates a straight line, although the degree of approximation is not as good for the series based on less than 100 chicks as it is for the larger series (Fig. 1). Grossly, the lines all possess the same slopes, but their intercepts on the comb-weight axis are different; similarly, separate plots for the 2 sexes

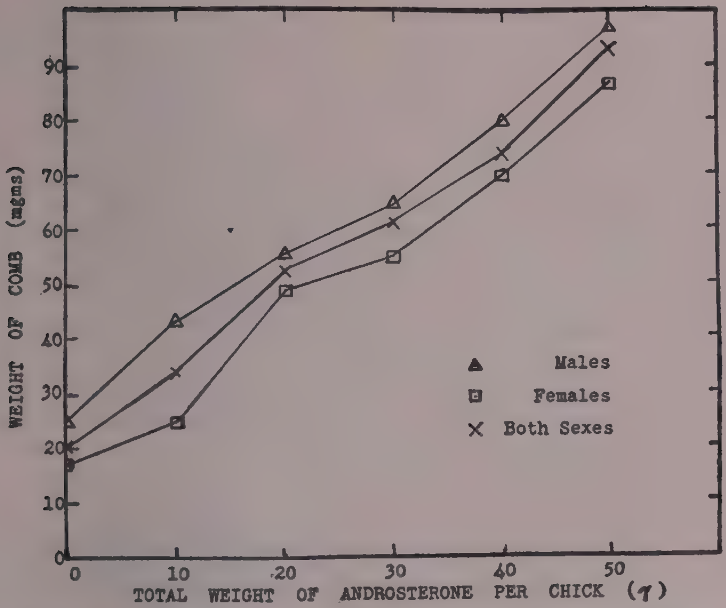

FIG. 1.

Relations between comb weight and androsterone dosage for a single series (S 18) of 150 chicks, uncorrected for control values.

in any one series manifest a higher intercept for males than for females—in conformity with the observations of Dorfman.[2] In other words, the graphs corresponding to the independent series or to different sexes of the same series appear to be distinct but parallel.

Consequently, all these graphs (including those for opposite sexes) tend to coincide if each series of data is corrected by subtracting from the mean comb weight for each group the corresponding value for the untreated chicks (controls) of the same series and sex. The identity of these corrected graphs is sufficiently close to indicate the existence of a characteristic curve for assay purposes; the fact that this curve is practically linear will make its use for these purposes a simple matter. The data for all 5 series of experiments, corrected in this way, have been combined into a single large series of 582 chicks, making adequate allowance for the number of chicks in each group. These combined plots—for separate and mixed sexes—are shown in Fig. 2.

Further efforts to increase the uniformity of the results obtained with small groups of chicks are now in progress. The final report will contain a detailed statistical analysis of the data.

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[2] Dorfman, R. I., and Greulich, W. W., *Yale J. Biol. and Med.*, 1937, **10**, 79.

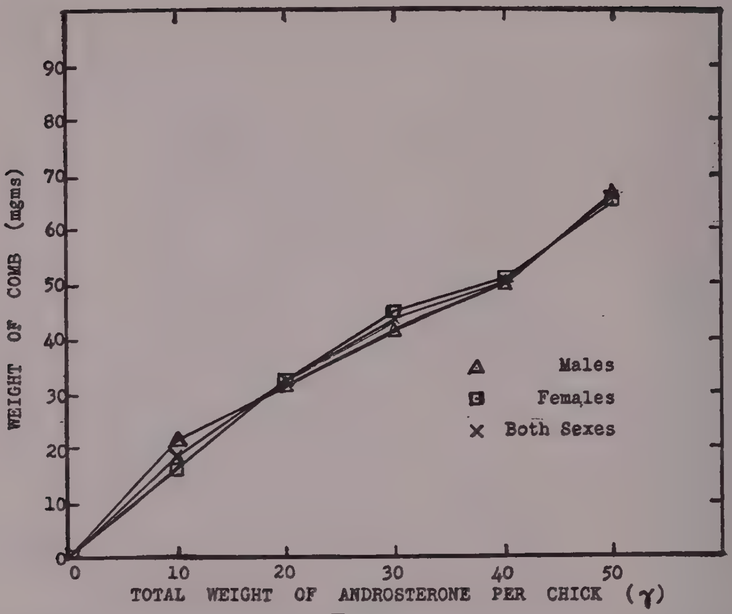

FIG. 2.

Relations between comb weight and androsterone dosage for all 5 series combined, of 582 chicks, corrected for control values.

We desire to express our thanks to Dr. Erwin Schwenk of the Schering Corporation of New Jersey for supplying us with the androsterone used in this investigation.

10039 P

Penetration of Radioactive Ions, Their Accumulation by Protoplasm of Living Cells (*Nitella coronata*).

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Exploratory experiments with K*, Na*, Rb* and Br*[†] reveal hitherto unknown steps of the process of penetration of these ions into living cells. Studies of plant cells with K*[1, 2, 3] have already shown that it is indispensable to separate the protoplasm from the sap, and to study both of these portions separately. Animal cells lack an obvious vacuole, but experiments with erythrocytes *in vivo* show that the absorption or removal of ions from the blood as a whole takes place. The amount of ions was deduced from the emission of β particles, using a Geiger-Müller counter and the ratio of the count to the known amount of the radioactive salt used.‡ When K*Cl is injected in a *ca,* isotonic solution into a rabbit (which has K rich erythrocytes) the K* disappears from the plasma mainly by excretion or absorption, but apparently enters the erythrocytes to approximate diffusion equilibrium but without conspicuous accumulation. When Na*Cl is injected neither loss from the blood nor accumulation of Na* takes place conspicuously. It is hoped that more significant data can be obtained by experiments *in vitro.*

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[*] This investigation has been supported by the Board of Research, and the radioactive elements have been provided by the Radiation Laboratory of this University.

[†] We shall refer to radioactive isotopes of elements as K*, Na*, etc., in contrast to the inactive elements, K, Na, etc.

Earlier experiments with *Nitella* show that K* does not accumulate to any great extent (*i. e.,* attain concentrations exceeding those of the immersion fluid) in the *sap* during about the first 10 hours. But during this period we can find pronounced accumulation of ions in the *protoplasm,* the concentrations being often 10 or 25 times as high in the protoplasm as in the immersion fluid. In this accumulation we can distinguish 3 or 4 phases, shown by K*: (1) a rapid intake of K*, usually complete within 1 or 2 hours, which may be an exchange of K* from the solution for K from the cell; (2) loss of K* from the protoplasm continuing for 2 to 4 hours, and possibly due to the slower entrance of other cations, replacing K* and K. This exchange is relatively slow because these ions have other properties (*e. g.,* Na) or a lower concentration (*e. g.,* H) in exchange for the K* already taken in. (3) Steady intake of K* possibly due to exchange of K* from the medium for metabolic products, *viz.,* weak electrolyte cations.

In one experiment 3 lots of cells were first exposed to 0.01 M KCl for 4 days, while one lot was not so treated. Then the control cells were exposed to 0.01 M K*Cl while the treated lots were exposed to 0.0033, 0.01 and 0.03 M K*Cl respectively. During Phase 1 the cells took in K* into the protoplasm in proportion to the K content of the cells as shown by the difference between the control

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[1] Brooks, S. C., *Trans. Faraday Soc.,* 1937, **33**, 1002.

[2] Brooks, S. C., *Am. Nat.,* 1938, **72**, 124.

[3] Brooks, S. C., *J. Cell. Comp. Physiol.,* 1938, **11**, 247.

‡ Experimental details are given in reference 3 in essentially the form used in the present work.

cells and similar cells which presumably took in K during the previous immersion in KCl. Furthermore, the amount of K* taken in by cells first treated and then exposed to 0.0033, 0.01 and 0.03 M K*Cl was at least approximately proportional to those concentrations. In contrast with this, during Phase 3, starting at different initial levels of concentration, there was no difference in the rate of intake. Penetration was independent of the external concentration of K*Cl and was dependent on forces put into play by the cell, presumably metabolic in nature.

A still later phase, with loss of K* from the protoplasm, can be distinguished as Phase 4. It occurred after 12 to 18 hours. This may result from injury, and was usually accompanied by increase of K* in the sap at this stage.

Similar experiments with *Nitella* previously immersed in NaCl instead of KCl and then in Na*Cl, showed that Phase I led to intake of Na* proportional to the external concentration of Na*Cl, as in the case of K*. But there is little difference between cells previously exposed to NaCl and not so exposed. We may conclude that Na is not taken in rapidly, although Na* can be rapidly exchanged for K or Na from the cell. After Phase 2 brought the concentration of Na* to a lower level, Phase 3 revealed almost no intake, confirming the low rate of entrance of Na.

Comparison of the rate of Na*, K* and Rb* during Phase 3, which may reflect the permeability of the protoplasm to different ions, yielded values of 0.001, 0.01 and 0.005 M/hour respectively. The data for Rb* were obtained under different conditions, but if we assume the validity of all the points, we may conclude that the penetrability of K>Rb>Na.

For Br* we find that under comparable conditions, (1) there is no Phase 1 exchange, possibly because there is no Br in the normal cell; (2) Br* may penetrate from a distilled water solution of KBr* (pH 6.6) at a rate of 0.006 M/hr, while K*Cl from a distilled water solution (pH 6.6) penetrates at a rate of 0.015 M/hr.

We have also tried single experiments on the effects of pH[1,3], of various compounds which may produce considerable increase of intake of K* during Phase 1, so that they cause accumulation in the protoplasm, up to about 20 times that in the external concentration, and of CN^- 5×10^4 M, which increases the K* concentration in the protoplasm as observed after 5 hours' exposure to K*Cl, while less K* gets into the sap at that time. The later stages show differences according to the pH, using 1×10^4 M buffers: phosphates at 5.75 and 6.68, and borate at pH 8.0.

10040 P

Gelation of Serum by Ethyl Alcohol.

Alfred Lewin. (Introduced by W. Antopol.)
From the Medical Clinic, University of Basle, Switzerland.

In the course of experiments on denaturation of serum* I observed the phenomenon of gelation of human sera following the addition of ethyl alcohol in higher concentrations. Gaté and Papacostas[1] described a similar serological reaction using formaldehyde. They offered this as a test for the diagnosis of syphilis but subsequently it was found to be of no value as such.

Principle of method: A known amount of ethyl alcohol (80%) is added to an equal amount of serum, shaken well, and the time determined in which the mixture turns into a gel. The gelation may at times be so complete that the tube may be inverted without the contents being spilled. The test-tubes used for the reaction must be of equal diameters when comparative studies are made.

Method: 0.2 cc of serum are measured into test-tubes 8 cm long, 7.5 mm internal diameter. Over the serum is then stratified 0.2 cc 80% ethyl alcohol. At the adjacent surfaces of these fluids a precipitate is formed. The tubes are then well corked and shaken vigorously until the mixing of the serum and alcohol is complete. This is recognizable by the milky white to yellowish white appearance which the solution assumes. The results are designated according to the degree of gelation which has taken place as follows: +++, complete; ++(+), almost complete; ++, strong; +(+), medium; +, weak; (+) beginning; —, none.

The test is carried out at room temperature. After 5 min., 30 min. and 3 hours the results are read; each tube is taken from the test-tube stand and is slowly tilted between thumb and forefinger at first to an angle of almost 90° and then, if the gelation is strong to complete, to an angle of 180°. While the conditions of this experiments are very simple, the differences in the intensity of gelation are very striking. In time the mixture coagulates to such a degree that one single large flocule is formed.

The time of gelation can be markedly influenced in several ways.

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* The work was begun under supervision of Professor H. Sachs at the Institute for Experimental Cancer Research at Heidelberg during the winter 1934-35, and continued in the institute of Professor R. Staehelin in Basle.

[1] Gaté and Papacostas, *Compt. rend. Soc. de Biol.*, 1920, **83**, 1432; *ibid.*, 1923, **89**, 1305.

The gelation is accelerated and intensified: (a) by increasing the concentration of the alcohol; (b) by raising the temperature of the mixture; (c) by inactivation of the serum (that is by heating ½ hour at 55-56° C); (d) by aging the serum.

Experiments on about 1500 sera of various origins were made, and the results, which will be reported in more detail in a later publication, are as follows:

1. The complete gelation in normal sera takes place after several hours. In certain cases however it does not occur even after days. 2. It is noticeable that positive Wassermann sera react like normal sera. 3. In most cases the value of the gelation was correlated with the sedimentation rate and only to a certain degree did the 2 reactions run parallel. Apart from normal sera there was no parallelism between the rate of the gelation and the sedimentation time. 4. Other serum reactions were also used for comparison. Takata-Staub positive sera in several cases showed a corresponding positive result to the gelation test. 5. At the first reading (after 5 min. at room temperature) the gelation was strong to complete in many cases of the following diseases: pulmonary tuberculosis, pleurisy, lobar pneumonia, Hodgkin's disease, cirrhosis of the liver, carcinoma.

The phenomenon described is a non-specific serum reaction which is striking in certain diseases. Its value as a differential diagnostic test remains to be determined by further studies.

10041

Failure of Nicotinic Acid to Prevent Nutritional Cytopenia in the Monkey.*

PAUL L. DAY, WILLIAM C. LANGSTON AND WILLIAM J. DARBY.

From the Departments of Physiological Chemistry and Anatomy, School of Medicine, University of Arkansas, Little Rock.

Day, Langston and Shukers[1] demonstrated that when monkeys (*Macaca mulatta*) were given a diet "deficient in vitamin G (B_2) and possibly deficient in less well-known organic substances which may be essential . . ." they developed a rapidly progressive blood dyscrasia characterized by anemia, leukopenia, loss of weight and death, and, in some cases, ulceration of the gums, anorexia and diarrhea. It was later shown[2] that when the diet was supplemented with yeast or a liver-stomach preparation the blood picture remained normal. Unpublished experiments have demonstrated that riboflavin did not appreciably alter the course of the disease.

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* Research paper No. 515, Journal Series, University of Arkansas. Aided by grants from the Committee on Scientific Research of the American Medical Association and the Ella Sachs Plotz Foundation.

[1] Day, Paul L., Langston, W. C., and Shukers, C. F., *J. Nutrition*, 1935, **9**, 637.

Funk and Funk[3] reported that nicotinic acid possessed vitamin activity. Recent experiments have shown that this substance is effective in the treatment of black-tongue in dogs[4,5] and pellagra in man.[6, 7] György[8] has reported that it prevented nutritional panmyelophthisis in rats.[9] The effectiveness of nicotinic acid in these nutritional disorders suggested its use in experimental anemia and leukopenia in the monkey.

In the experiments here reported the original monkey diet[1] was modified by the substitution of 0.01 g of ascorbic acid (Merck) daily for the orange juice, and the use of a more complete salt mixture.[10] Nicotinic acid (Eastman) was added in the form of a standard aqueous solution, which was mixed with the individual portions of the diet immediately before feeding. Detailed data on 2 of the monkeys are presented in Table I. Similar data were obtained in one other animal which also received 10 mg of nicotinic acid, and in 2 monkeys which received 50 mg daily. All 5 of the monkeys developed the characteristic syndrome of leukopenia and anemia and the condition terminated fatally in periods of time similar to the survival of monkeys receiving the deficient diet only. The blood picture of normal animals in our colony is presented elsewhere.[11] It is evident that the nicotinic acid was ineffective in pre-

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[3] Funk, C., and Funk, I. C., *J. Biol. Chem.*, 1937, **119**, xxxv.

[4] Elvehjem, C. A., Madden, R. J., Strong, F. M., and Woolley, D. W., *J. Am. Chem. Soc.*, 1937, **59**, 1767; *J. Biol. Chem.*, 1938, **123**, 137.

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[6] Spies, T. D., Cooper, C., and Blankenhorn, M. A., *J. A. M. A.*, 1938, **110**, 622, 766.

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[8] György, P., PROC. SOC. EXP. BIOL. AND MED., 1938, **37**, 732.

[9] György, P., Goldblatt, H., Miller, F. R., and Fulton, R. P., *J. Exp. Med.*, 1937, **66**, 579.

[10] Hubbell, R. B., Mendel, L. B., and Wakeman, A. J., *J. Nutrition*, 1937, **14**, 273.

[11] Shukers, C. F., Langston, W. C., and Day, Paul L., *Folia Hæmatol.*, 1938, in press.

TABLE I.

Blood Pictures of Monkeys Receiving Deficient Diet Supplemented with 10 mg of Nicotinic Acid Daily.

Date	No. of days on diet	Total white blood cells	Total lymphocytes	Total neutrophils	Total erythrocytes	Hemoglobin, g	Wt, g	Remarks
				Monkey No. 37 ♂				
10-7		10,500	6,830	2,730	5,040,000	13.1	3455	
10-21		8,700	6,180	2,260	5,385,000	10.1	3465	On diet 10-22-37
11-4	13	10,775	5,820	4,310	6,015,000	13.5	3135	
11-18	27	11,975	5,510	5,630	5,265,000	10.2	3095	
12-2	41	8,250	3,550	4,370	4,280,000	10.4	3075	Gums slightly injected; bleed easily
12-7	46	4,550	2,180	2,180	3,760,000	9.16	2940	
12-9	48	5,000	2,100	2,750	4,215,000	10.3	2850	
12-16	55	4,225	1,820	1,900	4,095,000	8.54	2740	
12-23	62	6,900	2,550	4,000	4,310,000	9.64	2570	Weak
12-28	67	8,675	1,300	7,370	4,105,000	7.66	2505	Eating diet satisfactorily
1-4	74	925	680	170	3,850,000	10.4	2280	Died
				Monkey No. 43 ♂				
1-19		18,950	8,900	8,720	4,965,000	11.8	2145	
1-26		15,050	7,680	5,720	5,725,000	10.5	2200	On diet 2-12-38
3-3	19	10,325	7,020	3,100	4,405,000	8.8	1920	
3-19	35	12,925	8,530	3,620	3,825,000	8.42	1945	
3-25	41	11,600	6,030	5,220	3,820,000	9.09	1940	
4-7	54	10,200	6,630	3,060	3,805,000	7.80	1970	Gums bleed easily
4-20	67	7,525	2,100	5,420	3,650,000	7.22	1815	Gums necrotic along margin
4-24	71	2,160			4,375,000	8.21	1580	Died

venting the blood dyscrasia. In view of the rapid disappearance of oral lesions in pellagrins treated with nicotinic acid, it is interesting to note that the substance failed to prevent the gum ulceration in the monkeys.

These data clearly indicate that monkeys require some factor (contained in yeast and liver extract[2,11]) in addition to those commonly recognized as components of the vitamin B complex. We do not interpret these results as showing that monkeys do not require nicotinic acid, but rather that this factor necessary to prevent nutritional cytopenia in the monkey is not identical with nicotinic acid. Furthermore, this syndrome in monkeys is not analogous to black-tongue in dogs or to that portion of the pellagra syndrome which is cured by nicotinic acid. Although Harris did not report blood findings in his experiments on the P-P requirement of the monkey,[12] the diet, weight curves, and the survival periods would suggest that his animals may also have been suffering from a partial deficiency of this hitherto undifferentiated factor. In view of the similarity of György's panmyelophthisis in rats and this nutritional cytopenia in monkeys, it is surprising that the one is prevented by nicotinic acid and that the other is not.

Summary. Five monkeys were given a diet which experience had shown would produce anemia, leukopenia and death. Three of the monkeys received daily supplements of 10 mg, and 2 received 50 mg of nicotinic acid. The nicotinic acid did not prevent the cytopenia or prolong life. It is evident that this syndrome in the monkey is not analogous to black-tongue in dogs or pellagra in man. We wish to propose the designation "vitamin M" for the hitherto undifferentiated factor which prevents this nutritional cytopenia in the monkey.

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[12] Harris, L. J., *Biochem. J.*, 1937, **31**, 1414.

10042 P

Decerebration in the Dog by Complete Temporary Anemia of the Brain.

Herman Kabat and Clarence Dennis. (Introduced by M. B. Visscher.)

From the Department of Physiology, University of Minnesota, Minneapolis.

In many investigations, it is desirable to perform experiments upon animals in the absence of anesthetics. The usual procedure is that of Sherrington, namely, to decerebrate surgically under ether, to stop the anesthesia, and to proceed at once with the experiment.[1] Under such conditions, profound shock and the depression due to the residual anesthetic agent throw some doubt on the reliability of the results. A more delicate method of decerebration has been devised for the cat by Pollock and Davis.[2] In this method, an anemic decerebration is produced by ligation of the basilar and carotid arteries under ether anesthesia. In the dog, the inaccessibility of the basilar artery makes this a difficult method.

It has been shown that the nerve cells of the brain stem are much more resistant to anemia than are those of the cerebral hemispheres.[3] It is therefore possible to produce extensive cerebral damage by a period of anemia which will allow the cells of the brain stem to recover. Guthrie, Pike and Stewart[4] and Gildea and Cobb[5] studied the effects of temporary occlusion of the 4 chief cerebral vessels in the neck under ether anesthesia, and were unable to predict the severity of the destruction produced by a given period of such occlusion. The variability in their results was due primarily to their failure to interrupt the flow in the spinal arteries, which is not constant from animal to animal. A further uncertainty must have resulted from variations in the depth of anesthesia just prior to the occlusion of the cerebral vessels.

We have devised a method of decerebration depending upon complete temporary anemia of the brain of the dog in the absence of anesthetics. A preliminary surgical procedure is used, consisting in removal of both laminæ and spine of the second cervical vertebra and ligation of both vertebral arteries. After an interval of one to 2 weeks, the dog is given one to 2 mg of atropin sulfate to prevent vagal cardiac inhibition, and placed, back down, on the table. A large blood-pressure cuff is wrapped around the neck, and the trachea intubated orally. By means of compressed air, the cuff is rapidly inflated to a pressure of 350 mm Hg and maintained at this pressure. This procedure causes sudden complete anemia of the head since not only the carotids and the cervical branches of the subclavians are occluded, but also the spinal vessels, by virtue of compression of the spinal cord.

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[5] Gildea, E. F., and Cobb, S., *Arch. Neurol. and Psychiat.*, 1930, **23**, 876.

Sudden complete anemia of the head is followed by unconsciousness. There is a period of asphyxial activity and panting for a short time. After several minutes, respiration stops, the animal becomes flaccid, and the lid wink reflex disappears. As soon as respiration stops, rhythmic artificial respiration is begun through the tracheal cannula. The heart usually beats rapidly and vigorously throughout the procedure, but must be followed closely for signs of vagal inhibition. After a period of anemia of 15 to 20 minutes, the pressure is released. Artificial respiration is continued until the dog begins to breathe spontaneously. The lid wink returns some minutes later. It is necessary to keep the animal in a constant-temperature box at 29°C.

We have kept a dog alive for 9 days after 19 minutes of complete anemia. He appeared comatose throughout, could not stand, but lay on whichever side he was placed, showing occasional rhythmic running movements of all 4 limbs together with high-pitched vocalization. The extensor tonus was only moderately increased. Temperature regulation was markedly impaired, and no change resulted from an injection of foreign protein which caused marked fever and panting in a normal control dog. There was no evidence of the presence of sensations of smell, vision, taste, hearing, or pain. The dog was unable to eat, and had to be fed by stomach tube. Such brain-stem reflexes as the light reflex, lid wink, swallowing, and vomiting could be elicited. The carotid sinus and aortic arch reflexes were readily obtained, and demonstrated a hyperactivity of the cardiac vagus. Arterial blood pressure was 130 mm Hg. There was a normal respiratory response to administration of 2.5% to 10% CO_2 in oxygen. No change was noted in the cerebral activity of this dog during the 9 days of observation.

The brains of several such animals are being studied histologically and subsequent reports will be made on this and other aspects of the investigation.

10043

Reticulocytogenic Effects of Liver Extract and Congo Red in Guinea Pigs under Reduced Oxygen Tension.

ARTHUR P. RICHARDSON AND WILLIAM DOCK.

From the Departments of Pharmacology and of Pathology, Stanford University School of Medicine, San Francisco.

Some investigators have claimed significant rises in reticulocytes after administration of potent liver extracts to guinea pigs,[1-6] while others have denied these claims.[7-10] Jacobson[11] suggested that the discrepancy in results could be explained by differences in diet. He believes that a diet of lettuce, carrots, and oats lacks some factor which is contained in liver extract. In support of this view, Gall[12] reported that the bone marrow of some of Jacobson's guinea pigs on this limited diet showed a densely cellular marrow with a preponderance of immature cells. Following the administration of liver to some of these animals, there was a marked depletion of the cellular content and accelerated maturation of the marrow elements. Inasmuch as the usefulness of guinea pigs for the bioassay of antipernicious anemia substances is controversial it appeared desirable to explore the reticulocytogenic action of liver in these animals under various conditions.

If, as some observers[1-6, 12] believe, the guinea pigs which have been on restricted diets and are "reactive" to liver extract have a retarded rate of maturation of the red cell series, they should respond slowly and inadequately to demands for increased rates of hematopoiesis. Guinea pigs in such a state should therefore have a less marked response to low atmospheric pressure than animals

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[4] Clark, G. W., and Coene, A. M., *Proc. Am. Soc. Biol. Chem.*, 30th Annual Meeting, 1936, p. 16.

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treated with liver extract containing the so-called "hematopoietic" fraction. Mermod, in some preliminary and unpublished experiments, observed no difference in the reticulocytosis and rise in red count of untreated reactive guinea pigs and those treated with liver when both groups were subjected to 10-12 days of 360-400 mm barometric pressure. This report confirms and extends her observations, and deals with the effect of Congo red and the bone marrow histology in such animals.

Methods. Twenty adult guinea pigs were used. All were fed on a diet of lettuce, oats, and carrots, or of lettuce, oats, and turnips,[9] during an experiment and for at least one month prior to injection of liver extract. Reticulocytes were counted at least every other day by the usual wet technic, using brilliant cresyl blue and sodium citrate. Blood was obtained by puncturing a small ear vein. Concentrated Liver Extract, Lilly (N.N.R.), was injected intramuscularly, using 1.0 cc per kg on 3 successive days, total 3.0 cc per kg. Congo red in 5% dextrose solution was injected intraperitoneally, using 50 mg per kg daily for 4 successive days, total 200 mg per kg.

Following a 3-day period of control counts of reticulocytes, the guinea pigs were put into a specially constructed steel tank (30 x 30 x 50 cm). Vacuum was produced by a Nelson vacuum and pressure pump. Pressure was maintained by a Saad valve so that air flowed through the chamber at an absolute pressure of 380 to 400 mm Hg., or about 76 to 80 mm partial pressure oxygen. The reduced pressure was maintained for 24 hours daily except for a short time each day for changing food and water, and making reticulocyte counts. The results obtained are presented as curves of average per cent changes in Figure 1.

Controls. When 5 normal animals, which had been on the experimental diet for one month, were subjected to such reduced pressure, the results obtained agreed practically with those of Gordon and Kleinberg.[13] In 2-4 days, the reticulocytes began to rise, reaching a peak in about 7-12 days, of from 8 to 14%. Then the percentage fell gradually and in 3-4 weeks the reticulocyte count approached the control (Fig. 1).

Liver Extract. After being one month on the experimental diet, 2 guinea pigs were injected with liver extract. One week after the first injection, when they should have given a reticulocyte response, they were put into the low pressure tank. The results in Fig. 1, which are labelled "Liver before Reduced Pressure," represent the average of 2 guinea pigs. It is seen that, before the period of low oxygen tension, these animals showed a slight reticulocytosis of 2.7%, presumably due to liver. It is clear, however, that this treatment did not affect the response of the bone marrow to chronic anoxemia. The peak in one animal was 10.2% reticulocytes, while in the other animal, 11.1%.

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[13] Gordon, A. S., and Kleinberg W., PROC. SOC. EXP. BIOL. AND MED., 1937, **37**, 507.

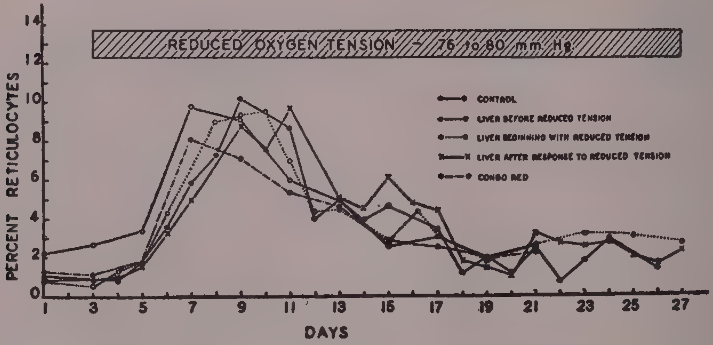

FIG. 1.

Comparative reticulocytogenic effects of liver extract and Congo red in guinea pigs under reduced oxygen tension.

Five guinea pigs were injected with liver extract and placed at once into the low pressure tank. The results in Fig. 1, labelled "Liver Beginning with Reduced Tension," represent the average of all 5 animals. The changes were practically the same as those in the controls, the peaks of individual responses varying from 9.5% to 11.4%.

Five guinea pigs were subjected to low oxygen tension for 2 weeks, or until the peak of the reticulocyte shower had passed, and the percentage of the reticulated cells had fallen nearly to the control level. At this point, the animals were injected with liver extract, and kept in the tank for 10 days. The average results labelled in Fig. 1 "Liver after Response to Reduced Tension," show that, under these conditions, liver extract was ineffective on the circulating reticulocytes.

Congo red. Since Congo red may cause remissions in active cases of pernicious anemia,[14, 15] the reticulocytogenic effect of this dye was tested under low oxygen tensions. Three guinea pigs were given, intraperitoneally, 200 mg per kg of Congo red, these injec-

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[14] Massa, M., and Zolezzi, G., *Klin. Wochnschr.*, 1935, **14**, 235.

[15] Mermod, C., and Dock, W., *Science*, 1935, **82**, 155.

tions being started at the same time the animals were put into the reduced pressure tank. The peak responses varied from 7.8 to 8.2%, *i. e.*, were somewhat lower than those of the controls (Fig. 1), the differences probably being without significance.

Bone Marrow. At the conclusion of the experiments most of the animals were killed and bone marrow from the lower vertebræ and femur was examined histologically. All the animals exposed to reduced oxygen tensions showed hyperplasia with very few fat vacuoles. While there was a slight decrease in eosinophiles, primitive red cells, and in megakaryocytes in the treated as contrasted with control animals, there was so much variation from one animal to another in the same group, or even from the femoral to the vertebral marrow of the same animal, that it was not possible, from the cells of the marrow, to distinguish the treated animals from the others.

These experiments prove clearly that guinea pigs, placed on diets which presumably render them reactive to liver extract, have no defect in the power of the marrow to form red cells and mobilize reticulocytes when the marrow is stimulated by arterial anoxemia due to low barometric pressure. Such exposure to low oxygen tension does not heighten the reticulocyte response of liver-treated animals over that of the controls, and instead of making the response to liver extract more striking than that under normal conditions, it entirely conceals any effect the liver may have on these animals.

It seems obvious that if there is a blood disorder in guinea pigs on the lettuce, carrot and oats diet, this is extremely mild since it results neither in anemia, nor in diminished response to hematopoietic stimulus such as anoxemia. At present it seems improper to speak of such animals as having an arrest in maturation of erythropoietic cells.

Conclusions. Guinea pigs subjected to reduced oxygen tensions showed prompt rises in reticulated red cells which reached a peak (8-14%), in 7-12 days. Then the reticulocytes decreased and in 3-4 weeks approached the control level.

Intramuscular injection of liver extract prior to, simultaneously with, or following, exposure to reduced oxygen pressure, had no demonstrable effects on the reticulocyte response; and the bone marrow of liver-treated animals could not be distinguished from that of untreated controls.

Congo red was also without effect on the reticulocyte response of guinea pigs under reduced oxygen tensions.

10044

Vitamin A Deficiency in Chicks Fed Purified Rations Containing Cod Liver Oil.*

H. R. Bird and J. J. Oleson. (Introduced by E. B. Hart.)

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Previous publications from this laboratory[1,2,3] have described a type of paralysis of nutritional origin in growing chicks characterized by ataxia, loss of equilibrium, staggering gait, and lack of growth. This condition has been ascribed to vitamin B_4 deficiency. More recently histopathological studies on such paralyzed chicks revealed certain lesions which were very similar to those occurring in uncomplicated vitamin A deficiency, in spite of the fact that these chicks were receiving rations which contained 2% of cod liver oil

TABLE I.
Occurrence of Leg Paralysis and Loss of Equilibrium in Relation to Supply of Vitamin A.

Basal ration	Supplement	No. of chicks	No. with leg paralysis	No. with loss of equilibrium
452	None	12	10	6
452	20 mg oleum percomorphum/week	15	10	0
452	0.4 mg carotene/week	8	3	0
452S	2% liver extract + 2% autoclaved liver residue + 0.4 mg carotene/week + irradiation*	8	5	0
452S	2% liver extract + 2% autoclaved liver residue + irradiation	4	2	4
452S	3% bakers' yeast + 2% cod liver oil + 20 mg oleum percomorphum/week	4	4	0
452S	3% bakers' yeast + 0.4 mg carotene/week + irradiation	3	3	0
452S	3% irradiated yeast + 0.4 mg carotene/week	4	3	0
459	None	8	4	4
459	20 mg oleum percomorphum/week	8	0	0

*Chicks were irradiated, where indicated, with a quartz-mercury vapor lamp for ½ hour periods 3 times per week.

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* Published with the approval of the Director of the Wisconsin Agricultural Experiment Station.

[1] Keenan, J. A., Kline, O. L., Elvehjem, C. A., Hart, E. B., and Halpin, J. G., *J. Biol. Chem.*, 1933, **103**, 671.

[2] Kline, O. L., Bird, H. R., Elvehjem, C. A., and Hart, E. B., *J. Nutrition*, 1936, **11**, 515.

[3] Elvehjem, C. A., Phillips, P. H., and Hart, E. B., Proc. Soc. Exp. Biol. and Med., 1937, **36**, 129.

of medicinal grade. This oil contained at least 1500 vitamin A units (U.S.P.X.) per g. These findings led to an investigation of the adequacy of this level of oil as a source of vitamin A in purified rations kept at room temperature. The data are given in the table.

Ration 452S had the following composition: dextrin 70, reprecipitated casein 18, salts No. I 5, brewer's yeast 2, and water extracted lung 5. Ration 452 contained in addition 2% each of liver extract, autoclaved liver residue, and cod liver oil. Ration 459 contained in addition to the ingredients of 452S 2% of liver extract, 8% of defatted peanut residue, 2% of cod liver oil, and 5% of grit. These additions were made at the expense of the dextrin.

The results were very striking when either Oleum Percomorphum or a suspension of carotene in water was administered to chicks receiving these rations. There was complete prevention of the loss of equilibrium and staggering gait. For the most part the chicks were able to survive the experimental period, and there was a slight improvement in growth in some cases. However, the paralysis and incoördination of the leg muscles was accentuated so that the chicks walked with a characteristic high stepping gait and stood with hocks thrust forward and toes stiffened and extended as though there were a hypertonicity of the extensors. Tremors were observed in some cases. Thus it appears that loss of equilibrium is not a part of the vitamin B_4 syndrome, but is caused by a complicating deficiency of vitamin A, and that vitamin B_4 deficiency is characterized chiefly by incoördination of the leg muscles.

It is the purpose of this paper not only to describe this type of paralysis as it occurs uncomplicated by vitamin A deficiency, but also to point out the need for extreme care to avoid vitamin A deficiency when working with purified rations kept at room temperature. In all previous experiments the cod liver oil was mixed with the ration at intervals of one week or less because of the known instability of vitamin A, but destruction appears to have occurred in spite of the precautions that were taken. Further studies on this supposed destruction have not been undertaken, but the iron and copper salts in the salt mixture may play a part. We have eliminated this difficulty in all present work by administration twice weekly of standardized vitamin A concentrates directly to all chicks on synthetic diets.

10045

A Failure to Find Virus III Infection in Rabbit Breeding Stocks.

JOHN M. PEARCE. (Introduced by Carl Ten Broeck.)

From the Department of Animal and Plant Pathology, The Rockefeller Institute for Medical Research, Princeton, New Jersey.

Rivers and Tillett[1,2] and Miller, Andrewes and Swift[3,4] had no difficulty in recovering Virus III by serial testicular passage in presumably normal laboratory-stock rabbits. The disease occurred in the 3d, 4th, 5th, or 6th passage in over 30% of trials. Fifteen to 20% of stock rabbits were either refractory to infection or contained neutralizing antibodies in their serum. No other record of the isolation of Virus III has been found. Recently, for use in some contemplated studies on this disease, it seemed desirable to obtain a new strain of the virus from spontaneous cases. The following report deals with that attempt.

Two groups of 8 rabbits of varying age were selected. The initial inoculum consisted of an emulsion of a spontaneous mammary rabbit-tumor. This was injected in 0.4 cc amounts in each testis of the first rabbit of each series. The rabbits were killed at intervals of 4 days, their testes excised under aseptic conditions, ground with sand and sterile salt solution, and injected in 0.5 to 1 cc amounts into the testes of the next of each series. In no instance was there any clinical or histological evidence of Virus III infection. It seemed probable that failure was due to the absence of the virus in the animals used in this laboratory, all of which come from the breeding stock developed by Dr. Wade H. Brown of The Rockefeller Institute. This supposition was confirmed by the fact that of a group of 90 from the same source which were inoculated at various times with Virus III, no rabbit failed to develop either clinical or histological evidence of infection.

Therefore a search was instituted for an infected stock. This was done by testing the sera of sample rabbits for neutralizing antibodies by their ability to inhibit growth of Virus III in tissue-cultures. The occurrence of the typical Virus III inclusion-bodies

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[1] Rivers, T. M., and Tillett, W. S., *J. Exp. Med.*, 1923, **38**, 673.

[2] Rivers, T. M., and Tillett, W. S., *J. Exp. Med.*, 1924, **39**, 777.

[3] Miller, C. P., Jr., Andrewes, C. H., and Swift, H. F., *J. Exp. Med.*, 1924, **40**, 773.

[4] Miller, C. P., Jr., Andrewes, C. H., and Swift, H. F., *J. Exp. Med.*, 1924, **40**, 789.

in the nuclei of the proliferating cells was used as a criterion for the presence of virus. Andrewes[5] had previously shown that the sera of animals recovered from the experimental infection prevented the appearance of inclusion-bodies and had correlated their presence with infectivity of the culture. We have confirmed this, and have demonstrated that the neutralizing power persists for at least 8 months after clinical recovery. Immune serum diluted 1:32 was found to be capable of preventing the formation of inclusion-bodies. The sera of the tested animals were used undiluted. The cultures were inoculated with the original strain of the virus isolated by Dr. Thomas Rivers and courteously furnished by him. As shown in Table I, 7 groups of rabbits from various sources—a total of 163 individuals—were examined. The groups came from the Brown breeding stock of The Rockefeller Institute at Princeton, New Jersey; from the breeding stock of the laboratories of The Rockefeller Institute in New York City; and from 5 independent rabbitries, 4 in the vicinity of Princeton and one in New York. The animals varied in age from 6 weeks to over 2 yrs. Approximately equal numbers of each sex were used. In the majority of cases they were of mixed breed, but one group of 20 was composed of 14 pure breeds. No wild rabbits were tested. The experiments were performed during the fall and winter months.

TABLE I.

Source	No. of rabbit sera tested in cultures	Approximate no. of rabbits in colony	% tested
Brown stock, Princeton	100	700	14
Rockefeller Institute stock, New York	20	300	7
Independent stock A	11	30	33
” ” B	8	50	16
” ” C	8	25	33
” ” D	8	75	10
” ” E	8	20	40
Total	163	1200	14

None of the 163 sera tested showed neutralizing antibodies. If their absence is evidence of freedom from infection, it would appear that Virus III in rabbits is not common. It is of interest that the animals from The Rockefeller Institute laboratories in both Princeton and New York originated in part from the same stock in which Rivers and Tillett, and Miller, Andrewes and Swift, found such a high incidence of infection in 1923.

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[5] Andrewes, C. H., *Brit. J. Exp. Path.*, 1929, **10**, 273.

10046 P

Complement Fixation with Insulin as Antigen.

Russell Richardson. (With technical assistance of Peggy C. Kostal.)

From the George S. Cox Medical Research Institute, University of Pennsylvania, Philadelphia.

In testing a hypothesis regarding insulin it was observed that certain human sera specifically fixed complement in the presence of the regular insulin as supplied to patient, in U80 strength; as well as pork, beef, and crystalline insulin.*

To determine the optimal amount of insulin to be used in the test a titration was set up according to the method of Boerner and Lukens[1] following that of Hooker[2] for the Wassermann antigen, using a serum which had previously shown marked fixation of complement with insulin. The result (Table I) is similar to that found when the Wassermann antigen is titrated. With too large or too small an amount of either antigen or serum the maximal fixation is not obtained.

TABLE I.
Titration of Insulin as Antigen with a Strongly Positive Serum.

Serum (cc)	Units of insulin used as antigen				
	25U	20U	15U	10U	5U
0.0063	—	+	+	—	—
0.0125	—	++	++	+	—
0.025	++	++	++	++	++
0.05	++	++	+++	+++	+++
0.1	++	++++	++++	++++	++++
0.2	++	++	++	+	+

When this optimal dose of insulin had been determined human sera were tested, in the following quantities—0.1, 0.05, 0.025, 0.0125, and 0.0063 cc; the usual controls were employed. Sera that gave complete or almost complete inhibition of hemolysis in one or more tubes were considered to be positive. A few sera were found in which there was a slight but definite inhibition of hemolysis, recorded as a doubtful reaction. Tests were repeated on the original serum and from 1 to 8 times on sera collected at intervals of from 1 week to 2 months. In all except 5 persons these reactions showed results similar to those found in the first test. The variations were of the same order as are commonly found when the Wassermann test is repeated on a given serum. The results are shown in Table II.

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* We wish to thank the Eli Lilly Company for the very generous supply of insulin furnished for this work.

[1] Boerner, F., and Lukens, M., *Am. J. Clin. Path.*, 1937, **7**, 33, 155.

[2] Hooker, S. B., *J. Immunol.*, 1927, **14**, 129.

TABLE II.
Complement-fixation Reaction with Insulin as Antigen.

	Positive	Doubtful	Negative	Total
Thyroid	4	2	5	11
Pregnancy	9	4	20	33
Diabetes	37	10	153	200
Other diseases	3	1	44	48
Normals	6	3	58	67

The normals in this table consisted of doctors, nurses, and members of the laboratory staff. They were apparently in good health but had not all had physical examinations. All were free from clinical signs of syphilis and 42 had negative blood-Wassermann reactions.

The thyroid-patients had been selected at random from the Thyroid Clinic and varied both in clinical condition and in the height of the basal metabolic rate.

The stage of pregnancy varied from the 3d to the 8th month and appeared to show no correlation with the reaction.

The diabetic patients were unselected. The group included balanced and unbalanced patients as well as those who were on diet alone and on diet with insulin. In all of these patients the Wassermann reaction was negative. No definite correlation was found between the clinical condition and the reaction.

The other diseases consisted of groups of from 5 to 10 patients each with a diagnosis of carcinoma, tuberculosis, allergic condition, acute inflammation, chronic inflammation, some kind of blood disease, or syphilis with a positive blood-Wassermann reaction. Seven sera giving positive Wassermann reactions were negative with insulin; the Wassermann was negative in 52 insulin-positive sera tested for that reaction. In no serum were the Wassermann and insulin reactions both positive. Two insulin-positive sera did not react with insulin that had been inactivated by treatment with alkali.

It would then appear that there is present in the blood of certain persons a substance that is able to combine with insulin and thereby fix complement. Its nature and the degree of specificity of its action with insulin are not yet defined.

10047

Effect of Extract of Anterior Pituitary upon the Life-Span of Castrate-Adrenalectomized Cats.

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and J. A. Morrell.

From the Biological Laboratory, Princeton University, and Research Laboratory, E. R. Squibb & Sons, New Brunswick, N. J.

The writers have reported that a commercial extract of the anterior pituitary prolongs the life-span of bilaterally adrenalectomized cats, but is without effect upon dogs, at any rate in the dosage employed.[1] Two of the 12 animals studied were castrate males. One lived 25 days and succumbed to feline distemper; the other survived 47 days. It was deemed worth while to study the effect of A.P. extract upon the life-span of a larger group of castrate-adrenalectomized cats.

Strong, vigorous cats were used. They were given a vermifuge, and a few days later the gonads and right adrenal were removed under nembutal anesthesia. They were then kept in the laboratory for 2 weeks to insure adjustment to laboratory conditions. The animal room was thermostatically regulated. The left adrenal was extirpated under ether and for 2 days following operation the cats received cortical hormone. If the animals were eating full rations at this time cortical hormone was withdrawn and A.P. extract substituted. The experiment began the day cortical hormone was discontinued.

The A.P. extract used was prepared by a modification of the method of Van Dyke and Wallen-Lawrence[2] and is an alkaline aqueous extract of cattle pituitary made by Squibb & Sons. It contains appreciable amounts of the various pituitary factors. We used freshly prepared non-preserved material since the cats appeared to be sensitive to the merthiolate present in the commercial preparation.

The diet consisted of 175 g of canned salmon plus 50 cc of milk daily. This was changed to fresh raw fish, liver and kidney whenever the appetite became capricious. The essential data regarding the effect of A.P. extract upon the life-span are shown in Table I.

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* E. R. Squibb & Sons Fellow.

[1] Swingle, W. W., Parkins, W. M., Taylor, A. R., Hays, H. W., and Morrell, J. A., *Am. J. Physiol.*, 1937, **119**, 675.

[2] Van Dyke, H. B., and Wallen-Lawrence, Z., *J. Pharm. and Exp. Therap.*, 1930, **40**, 413.

TABLE I.
Effect of A.P. Extract* on Life-span of Castrate Bilaterally Adrenalectomized Cats.

Cat No.	Wt, kg	Sex	Survival period days	Cat No.	Wt, kg	Sex	Survival period days
1	4.02	♂	30	11	3.45	♂	11†
2	3.35	♂	23	12	3.45	♂	9
3	2.65	♂	15	13	2.25	♀	5 T
4	4.50	♂	14 T	14	3.43	♂	8 T
5	2.82	♀	15 T	15	3.26	♂	7†
6	3.80	♂	22	16	2.79	N.C. ♂	40
7	3.50	♂	28	17	2.58	N.C. ♂	49
8	3.10	♀	14	18	2.55	N.C. ♂	33
9	3.05	♂	31	S^1 6	2.0	♂	25
10	3.48	♂	10	S^1 10	2.10	♂	47

*Daily dose = 1 cc/kg. N.C. = Non-castrate. S^1 = Castrates of earlier series. † = Sacrificed—respiratory infection. T = Terminal hypoglycemia.

Of a total of 17 castrate-adrenalectomized cats, 11 showed definite prolongation of the life-span and 6 did not. Several cases in the short-survival series were complicated by respiratory infections. The average life-span of the group (including 2 castrates of an earlier series[1]) was 18.4 days. The survival period ranged from 5-47 days. A group of 3 bilaterally adrenalectomized but non-castrate male cats were given daily injections of A.P. extract in comparable doses in order to compare the effect of the extract in animals with and without sex glands. The average life-span of this small group was 40.6 days. In the earlier work on the non-castrate male and female cat injected with A.P. extract a series consisting of 10 animals were studied.[1] One animal survived 150 days and died of adrenal insufficiency 4 days after the A.P. extract was discontinued. Excluding this individual (since the life-span was exceptionally prolonged), the average survival for the remaining 9 non-castrates was 24.8 days. However, if the 3 non-castrates of the present series are included with the non-castrates previously described (9 animals) the average survival of the 12 individuals on A.P. extract is 32.7 days. Thus it seems clear that the non-castrate A.P. injected adrenalectomized cats survive longer than similarly injected but castrated animals (av. 18.4 days). The sex of the animal does not appear to be important in so far as the effect of the A.P. extract is concerned.

Although the A.P. extract prolongs the life-span of both castrate and non-castrate cats it by no means substitutes for the adrenal cortical hormone. The animals (especially the castrates) soon become sluggish, vigor decreases, the hair becomes rough and shaggy and tends to fall out, the appetite is capricious and all exhibit a slowly progressing cachexia. The long-surviving cats present a perfect picture of an animal suffering from chronic adrenal insufficiency. Terminal hypoglycemia is not uncommon in those individuals showing marked cachexia.

The explanation of the ameliorative effect of A.P. injections is not clear and this is especially true of the castrate animals. Since the A.P. extract contained some of the adrenotropic factor it is possible that microscopic cortical rests might have been stimulated sufficiently to elaborate enough of the vital hormone to maintain life. Although we have diligently searched for such accessories at autopsy none have been observed.

There can be no question but that the hormone of the corpus luteum ameliorates the symptoms of adrenal insufficiency in certain animals. This is well shown by the effects of pseudopregnancy upon the life-span of the adrenalectomized dog and ferret.[1,3,4] Collings[5] working in this laboratory has likewise demonstrated the striking effect of experimentally induced pseudopregnancy upon the life-span of the adrenalectomized cat.

Steiger and Reichstein[6] report that 21-oxy-progesterone has definite cortical hormone activity on adrenalectomized dogs and rats. The close similarity between progesterone and corticosterone[6] and the fact that 21-oxy-progesterone prolongs the life-span of adrenalectomized animals, apparently offers an adequate explanation for the effect of pseudopregnancy (which represents the corpus luteum phase of the cycle) upon the survival of the adrenalectomized dog, ferret and cat. Apparently the organism lacking adrenals has the ability to convert progesterone into corticosterone or related substances.

However, this does not explain the effect of A.P. extracts upon the life-span of the adrenalectomized male cat but suggestive evidence does exist. Thus Callow and Parkes[7] demonstrated the presence of appreciable quantities of progesterone in the testes of mammals. According to them, bull testes contain the equivalent of 1 mg of progesterone per 1400 g of tissue. It seems not unreasonable to assume that the testis of cats likewise contains progesterone and the daily injection of A.P. extract may stimulate the gonads to produce

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[3] Rogoff, J. M., and Stewart, G. N., *Am. J. Physiol.*, 1928, **86**, 20.

[4] Gaunt, R., and Hays, H. W., *Anat. Rec.* (Suppl.), 1938,**70**, 29.

[5] Collings, W. D., unpublished work.

[6] Steiger, M., and Reichstein, T., *Nature*, 1937, **139**, 925.

[7] Callow, R. K., and Parkes, A. S., *J. Physiol.* (Proceedings), 1936, **87**, 16.

excessive amounts which in time would be converted into corticosterone or related substances having cortical hormone-like activity. The fact that the A.P. injected non-castrate adrenalectomized cat is easier to maintain and survives longer than the similarly treated castrate fits well with this viewpoint. But the fact that the castrates also react to A.P. injections offers difficulties, unless it be assumed that other tissues besides the gonads can also form progesterone or related substances as a result of A.P. treatment.

Although the writers believe that the various factors enumerated may and probably do enter into the explanation of the ameliorative effect of A.P. extract on adrenal insufficiency in the cat, there remains another factor which we regard as important. This is the effect of A.P. extract upon the appetite, ingestion and digestion of foodstuffs, and the absorption and utilization of carbohydrate in these animals.

10048 P

Influence of Posture on Skin and Subcutaneous Temperatures.

H. S. MAYERSON AND L. A. TOTH.

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Previous experiments[1] showed that the cardiac output in the sitting and standing positions was less than in the recumbent. They also indicated the probability that the former positions facilitate the return of a disproportionately large amount of blood from the short systemic circuits, although the amount from the extremities and the splanchnic area is decreased. The present report deals with observations on blood flow, particularly in the extremities, as measured by surface and subcutaneous temperatures.

Eight adult subjects with normal cardiovascular systems have been systematically studied. Temperatures in various parts of the body were determined by means of surface and non-conducting hypodermic thermocouples in a potentiometric circuit so arranged as to permit rapid and accurate consecutive readings.[2] The subcutaneous thermocouples were inserted to a depth of from 2 to 4 mm. Passive change of position from the horizontal to an angle of 75 degrees was achieved by a tilt table, the subject being held in place by supports over the iliac crests. This method of support eliminated the necessity of a foot-rest, objectionable because of the tendency of subjects to carry and shift enough weight on their lower extremities to aid in the venous return through muscle action. The subjects remained at 75 degrees for from 14 to 40 min, being returned to the horizontal position when they complained of being uncomfortable or became faint. Room temperature was maintained relatively constant during each experiment by means of fans and heaters.

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[1] Sweeney, H. M., and Mayerson, H. S., *Am. J. Physiol.*, 1937, **120**, 329.

[2] Foster, P. C., *J. Lab. and Clin. Med.*, 1936, **22**, 68.

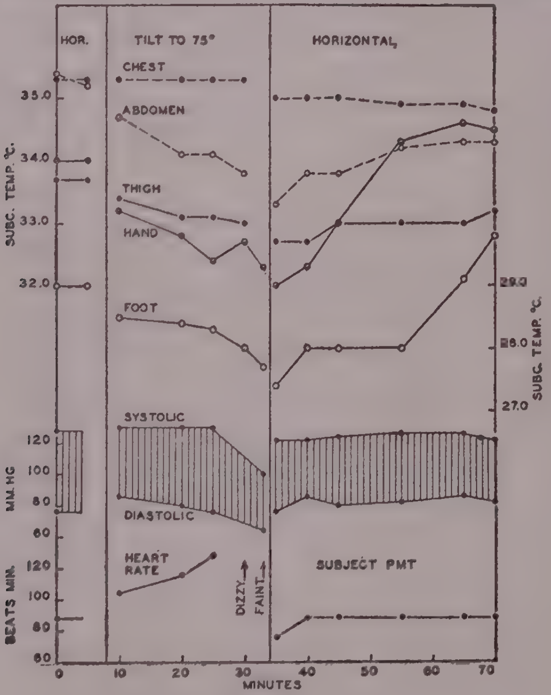

Fig. 1.

A typical experiment is charted in Figure 1. In addition to the usual surface to subcutaneous temperature gradient, both temperatures showed gradients from toes to forehead when the subjects were in the horizontal position. When tilted to 75 degrees, there was, in general, a progressive decrease in the temperatures of all parts of the body measured, being most marked in the extremities. The lowest values were usually reached at the end of the tilt period, or within one to three minutes after the return to the horizontal in those instances where the tilt period was terminated by fainting. In the majority of the experiments the temperatures returned to or were approaching their original levels in from 15 to 60 min after the subjects were returned to the horizontal position.

In 4 experiments a heating pad was wrapped around the left foot, thus reflexly dilating the arterioles of the right foot. Temperature measurements were made on the right side of the body. Such an experiment is charted in Figure 2. The results of these experiments indicate that arteriolar vasoconstriction is largely, if not entirely responsible for the decrease in temperature observed on tilting from the horizontal to the 75 degree position.

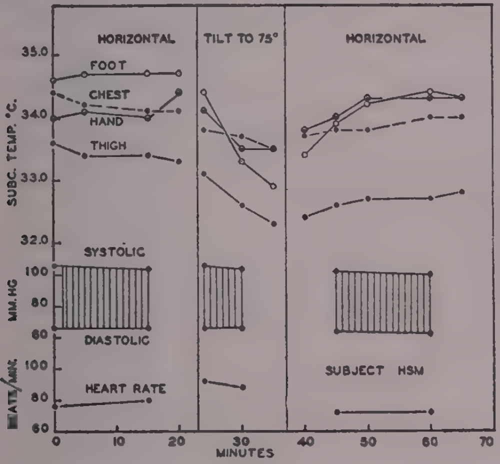

Fig. 2.

Three of our subjects showed no signs of discomfort when tilted for periods as long as 34 min, while 4 consistently showed signs of syncope within this period. One subject developed syncope on several occasions but not on others. The inability to tolerate the upright position could not be correlated with the changes in surface or subcutaneous temperatures. The similarity of our results with those of Weiss, Wilkins and Haynes[3] suggests the possibility that the development of syncope in the upright position is related to the amount of venous dilatation and pooling in the extremities which accompany the arteriolar constriction and reduction of blood flow.

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[3] Weiss, S., Wilkins, R. W., and Haynes, F. W., *J. Clin. Invest.*, 1937, **16**, 73; Wilkins, R. W., Haynes, F. W., and Weiss, S., *J. Clin. Invest.*, 1937, **16**, 85.

10049 P

Studies in Skin Distensibility.

William A. Sodeman and George E. Burch. (Introduced by J. H. Musser.)

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Heretofore there has been no simple adequate quantitative method for the estimation of skin distensibility. The importance of such data in an understanding of the pathologic physiology of edema and in an objective estimation of the results of therapeutic procedures in scleroderma is self-evident. The following method has proved simple and satisfactory. Two 4.8 mm bakelite cubes were sealed to the skin with collodion at a distance of 5 cm from each other. A steel spring caliper of known calibration was articulated at its tips with the bakelite cubes and when released spread the cubes apart with a force of approximately 100 g. The distance between the cubes was then measured and the distension expressed in mm of stretch per cm of skin per 100 g of force.

Determinations have been made in 13 normal subjects for the pretibial area, dorsum of the foot, abdomen, volar surface of the forearm and the dorsum of the hand, and the mean values were found to be 0.31, 0.59, 2.07, 0.93 and 1.34 mm/cm/100g, respectively. All females were nulliparous. The skin was found to be less distensible in the lower extremities, varying inversely with the tissue pressure.[1] This regional variation appears

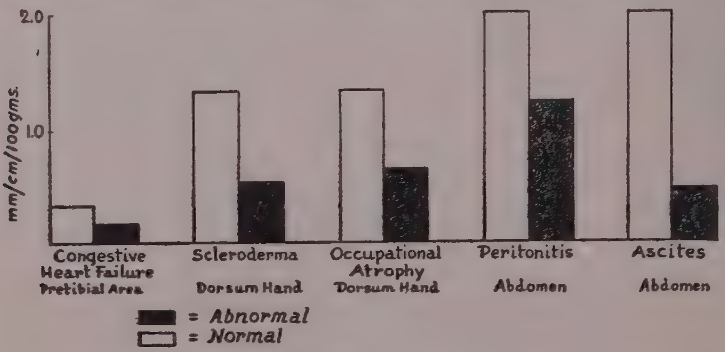

Fig. 1.
Mean skin distensibility values in normal and abnormal subjects.

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[1] Burch, G. E., and Sodeman, W. A., *J. Clin. Invest.*, 1937, **16**, 845.

to be of physiologic significance in the prevention of edema of the feet on assuming the erect position. Repeated determinations showed comparable results from day to day.

Forty-three determinations have been made on 21 patients with the following states: scleroderma 4, occupational atrophy 3, ascites 4, peritonitis 2, edema 8 (cardiac 6, venous obstruction 1, pernicious anemia 1). Comparison of the mean values in the patients with those in the normal subjects is illustrated in Figure 1. In scleroderma a diminution in distensibility was found to parallel the severity of the disease. Values for the dorsum of the hand ranged from 0.47 to 0.55 (mean 0.50), contrasting with the normal range of 0.66 to 2.04 (mean 1.34). This method is being used to follow quantitatively the progress of the disease and to evaluate therapeutic procedures.

In congestive heart failure the distensibility was found to decrease as the edema increased, reaching, in one patient, the limit of distensibility. Similar findings occurred in other types of edema.

Summary. A simple method has been devised for the measurement of skin distensibility. Its application to the study of the physiology of the skin in edema and abdominal swellings is being made. Its importance as an objective method in following, quantitatively, the progress of scleroderma and other skin changes, as well as objectively evaluating the therapeutic procedures applied to these diseases is evident.

10050

The Ammonia Mechanism in Alkalosis Due to Overventilation.

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It has been taught[1] that production of ammonia by the kidneys is increased for excretion of acid which would otherwise carry off essential basic elements; and that ammonia formation is suppressed in states of alkalosis when it is desirable for the body to be rid of excess fixed base. My own observations[2,3,4] have convinced me that the ammonia mechanism is not concerned with conservation of fixed base, but that it does serve to protect the urinary passages from the acid secretion formed within the renal tubules.

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[1] Peters, J. P., and Van Slyke, D. D., Quantitative Clinical Chemistry, Vol. I, Interpretations, Williams and Wilkins, Baltimore, 1931, p. 372.

[2] Briggs, A. P., *Arch. Int. Med.*, 1932, **49**, 56.

[3] Briggs, A. P., *J. Biol. Chem.*, 1934, **104**, 231.

[4] Briggs, A. P., *Arch. Int. Med.*, 1937, **60**, 193.

In the present contribution data associated with the development and recession of alkalosis from forced breathing are presented for inspection from the two viewpoints.

Respiration was accelerated sufficiently so as to maintain slight sensations of giddiness and numbness of the lips: just a low grade alkalosis. Urines were collected before, during and after the alkalosis period. Acids and bases were determined by familiar methods used in a previous publication.[4] Since a diuresis develops with overventilation and because a diuresis tends to waste fixed base, water was taken during the fore and after periods in sufficient quantities so that urine volumes were similar.

TABLE I.
Effect of Overventilation on Excretion of Ammonia and Excess Fixed Base.

Exp. I	Duration hr	Vol. urine cc	Cl. m.Eq.	SO_4 m.Eq.	PO_4 m.Eq.	Total acid m.Eq.	Total fixed base m.Eq.	Fixed base excess m.Eq.	NH_3 m. Eq.	pH
Before	1.0	135	13.78	1.65	0.79	16.22	17.60	1.38	1.67	5.8
During	1.0	102	14.76	1.16	0.70	16.62	27.50	10.88	0.35	7.5
After	1.0	220	8.04	1.15	0.59	9.78	14.26	4.48	0.62	6.6
II										
Before	1.0	365	6.66	0.50	1.87	9.03	10.94	1.91	0.72	6.4
During	1.0	305	5.57	0.88	1.80	8.25	15.25	7.00	0.62	7.3
After	1.0	185	5.66	0.84	1.33	7.73	9.35	1.62	1.30	6.5
III										
Before	2.0	508	23.08	0.96	1.72	25.76	27.52	1.76	2.50	6.5
During	2.0	620	19.10	0.63	1.67	21.40	41.90	20.50	1.06	7.5
After	2.0	470	10.84	0.89	1.26	12.99	14.37	1.38	4.12	5.9
IV										
Before	1.5	276	17.88	1.71	0.38	19.97	21.32	1.35	1.54	6.1
After	1.5	320	16.40	1.21	0.56	18.17	26.13	7.96	0.60	7.2

The results, in the accompanying table, show ammonia production to be at a low level in the control periods. Since there is little change in the excretion of ammonia with forced breathing the ammonia mechanism is not an important factor in expulsion of the conspicuous increment of excess fixed base, which is to be seen for this period. Comparing the alkalosis periods with the preperiods, the average increase in excess fixed base is 9.91 m.Eq. to a drop of 0.48 m.Eq. for ammonia.

This observed lack of proportionality might have been predicted from previous work on alkalosis of overventilation: similar slight changes in production of ammonia were observed by Grant and

Goldman[5] with the development of severe alkalosis; and the relatively large increment of excess fixed base which may be excreted in this state, up to 30 m.Eq. per hr, was shown in the study of McCance and Widdowson.[6]

It is of some interest to observe that the excretion of excess base is not due to diminished excretion of chloride. McCance and Widdowson similarly observed no drop in excretion of chloride with the development of alkalosis. The prompt excretion of a strongly alkaline urine with alkalosis may however be very readily accounted for by assuming that a state of alkalosis inhibits tubular resorption of base bicarbonate, a suggestion implied by Davies, Haldane and Kennaway.[7]

In the after periods, with removal of the inhibition of alkalosis, resorption of fixed base is more nearly complete; the urine again becomes slightly acid, and production of ammonia is slightly accelerated. The average increment of ammonia, however, is only 1.34 m.Eq. compared with a decrement of 10.31 m.Eq. for fixed base. Thus we have another instance showing lack of proportionality between ammonia production and conservation of fixed base. The comparison of fluctuations of ammonia production with fluctuations of excess fixed base is most favorable to the base regulatory theory; it would perhaps be more proper to compare ammonia excretion with total fixed base resorption. When we stop to calculate that something like a thousand m.Eq. of fixed base per hr appears to be resorbed by the tubules, the 2 or 3 m.Eq. of ammonia per hr produced under ordinary circumstances at once becomes insignificant; the diminished production with alkalosis scarcely less so, and the 5 or 10 m.Eq. per hr, produced with administration of strong acid, scarcely more so.

From my viewpoint, ammonia secretion in the different periods is accounted for as follows: the small quantities found for the alkalosis periods may be derived in part from the glomerular filtrate and in part from unremoved urine of the preperiod. The slightly greater quantities of ammonia for the control periods presumably results from the stimulation of undetermined organic acid, which the pH values indicate to be present in quantities greater than those calculated for excess fixed base.

Summary. A comparison has been made of fluctuations of ammonia secretion and fixed base excretion associated with the development and recession of alkalosis from forced breathing. The results appear to be out of harmony with the base regulatory theory of ammonia production.

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[5] Grant, S. B., and Goldman, Alfred, *Am. J. Physiol.*, 1920, **52**, 209.

[6] McCance, R. A., and Widdowson, E. M., *Proc. Roy. Soc.*, Series (B), 1936, **120**, 228.

[7] Davies, H. W., Haldane, J. B. S., and Kennaway, D. M., *J. Physiol.*, 1920, **54**, 32.

10051

Automaticity of Central Neurones After Nicotine Block of Synapses.

B. LIBET AND R. W. GERARD.

From the Department of Physiology, University of Chicago.

The discovery that central neurones manifest a rhythmic electrical potential in the absence of deliberate sensory stimulation suggested that these cells possess an automatic beat homologous to that of the cardiac nodal cells. The possibility of nerve impulses, either from receptors or injured nerves or from closed circuits of interneurones* with long maintained activity, had not, however, been excluded. The persistence of a marked potential rhythm in the frog's olfactory bulb for hours after its complete removal and at a time when its only neural connections—the olfactory nerves and cerebral hemispheres—were electrically dead[1] seemed to eliminate any action of impulses from receptors or injured neurones. Since, however, stimulation of the olfactory nerves of such a preparation increases the bulb potentials or even reinitiates them after they have stopped and since this enhancement may persist for many minutes or hours following a brief stimulation[2] the existence of trapped impulses in closed circuits remained a definite possibility. This has been urged especially by Lorente de No.[3] In the present experiments it is shown that when synaptic conduction is blocked by nicotine the rhythmic slow potential waves of the isolated bulb are increased rather than abolished. It may be concluded, therefore, that the activity rhythm originates in single neurones and is immediately independent of any maintained or recurrent bombardment by conducted nerve impulses.

Nicotine has long been used to block synaptic transmission in autonomic ganglia[4] and is also known to exert a curarizing action.[5,6] We have found no reference, however, to a similar effect on central synapses, though Langley[7] reported some experiments indicating its occurrence. The following facts evidence such a central blockade at all synapses tested:

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* This word is suggested as a condensation of "internuncial neurones" parallel to Sherrington's use of "motoneurone."

[1] Gerard, R. W., and Young, J. Z., *Proc. Roy. Soc. B*, 1937, **122**, 343.

[2] Libet, B., and Gerard, R. W., *Am. J. Physiol.*, 1938, **122**, 128 (P) and unpublished.

[3] Lorente de No, R., *Am. J. Physiol.*, 1935, **113**, 505.

1. Local application of 0.1% nicotine to the exposed spinal cord in the frog abolishes in 15 min. cross reflex responses to stimulation of the central end of a cut sciatic. Peripheral sciatic stimulation remains effective. Langley[7] published a similar observation. Even after strychninization, reflex block is produced by nicotine. Stronger nicotine (0.5%) abolishes motor responses to electrical stimulation of the cord itself though the lower concentrations do not.

2. Local application of 0.5% nicotine to the exposed optic lobes in a freshly amputated frog head abolishes the wink reflex elicited by touching the eye or nostril.

3. This same application eliminates, within 6 min, visual action potentials in the optic lobes, evoked by shining light in the eyes, while leaving unchanged the action potentials in the optic nerve. It also prevents the changes in olfactory bulb potentials otherwise produced by stimulation of the olfactory nerves.

4. Nicotine (2.5 mg per kilo) blocks tactile action potentials in the thalamus and somaesthetic radiations in the cat, evoked by touching hairs of a contralateral leg, within 4 min of its intravenous injection. At this concentration, the nicotine effect begins to wear off in 20 min and electric responses are again normal in an hour.

5. When the curarizing action of nicotine is prevented by clamping the vessels to the cat's leg, crossed and uncrossed spinal reflexes to it are abolished a few seconds after intravenous injection of the drug, when the motor nerve still gives good responses. Similar results are obtained for jaw and wink reflexes from the brain stem. Convulsive motor discharges may accompany synapse block.

6. Phrenic nerve action potentials continue after nicotine has abolished the other responses mentioned (see also [6]). Bursts continue at the usual frequency and intensity but are no longer spaced at regular intervals. Asphyxia still increases these discharges and eventually abolishes them. Stronger nicotine (10 to 13 mg per kilo) stops phrenic discharges while leaving cortical potentials slower (2 to 4 rather than 6 per second) and more regular than before.

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[4] Langley, J. N., and Dickinson, W. L., *Proc. Roy. Soc. B*, 1889, **46**, 423.

[5] Rosenthal, *Centralb. f. Medicin. Wissensch.*, 1863, **1**, 47.

[6] Gold, H., and Modell, W., *J. Pharm. and Exp. Therap.*, 1936, **57**, 310.

[7] Langley, J. N., *J. Physiol.*, 1901, **27**, 233.

It seems clear, therefore, that the phrenic nerve cells can discharge nerve impulses when afferent connections are blocked—presumably even synapsing ones from the medullary respiratory center.† Similarly, in the isolated olfactory bulb the neurones maintain their rhythm in the absence of impinging nerve impulses, for the rhythm is preserved, actually enhanced and made more regular, even after half an hour's soaking in 0.5% nicotine. Since large regular waves from a cell mass are possible only with considerable synchronization of individual units, this interaction is also possible in the absence of transmitted nerve impulses.

The chemical and physical control of the activity of the single neurone and of the coördination of the group has been discussed[8,9] and additional studies on the frog (Libet and Gerard) and cat (Dubner and Gerard) will soon be published.

We are indebted to Mr. O. Sugar for assistance in some of the cat experiments.

10052 P

Preparation of Cocarboxylase.

HENRY TAUBER.

From the Research Laboratory of the McLeod Infirmary, Florence, South Carolina.

From the reaction mixture which I have recently described[1] the synthetic cocarboxylase (thiaminpyrophosphate) may be obtained in crystalline form by the following procedure: 500 mg sodium pyrophosphate are placed in a Pyrex test-tube and heated until all of the water of crystallization is removed. One cc of orthophosphoric acid (cp 85%) is placed in another large Pyrex tube and heated until a slight amount of solid deposit forms on the side of the tube. Then the pyrophosphate is added and the mixture gently heated until solution takes place. After cooling 500 mg vitamin B_1.HC1 are added. The tube is placed in an oil bath of 155°, kept there for 3 min and constantly stirred. Then the tube is removed and after cooling the solid mass is dissolved in 10 cc of cold water. Cold saturated $Ba(OH)_2$ solution is added until no more precipitate forms and the solution is just commencing to turn yellow. The precipitate is centrifuged off and the supernatant fluid is decanted. The precipitate is extracted 4 times for 5 minutes with 40 cc of cold water. All 5 supernatant fluids are united and after cooling 3% H_2SO_4 is added to slight blue reaction of congo red paper. The $BaSO_4$ is centrifuged off and discarded. The Ba-free solution is concentrated to 30 cc in vacuum at 25°. It is cooled in ice water and 15 volumes of a mixture of 1 part of absolute alcohol and 2 parts of ether are added which precipitates the cocarboxylase in the form of microscopic needles. Sometimes a gummy mass will form which turns into long macroscopic needles on short standing in the cold. The preparation obtained on 6 recrystallizations from the alcohol-ether mixture, redissolved each time in 10 cc of N/5 HCl, and dried in vacuum is readily soluble in water and is free of inorganic salts.

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† The alternate possibility, that these synapses remain functional, is being further explored.

[8] Gerard, R. W., *Cold Spring Harbor Symposia*, 1936, **4**, 292.

[9] Blake, H., and Gerard, R. W., *Am. J. Physiol.*, 1937, **119**, 692.

[1] Tauber, H., *J. Am. Chem. Soc.*, 1938, **60**, 730.

This synthetic preparation is practically as active as natural cocarboxylase[2] (very kindly furnished by Professor Lohmann). Phosphorus and thiamin content, however, indicate that my cocarboxylase still contains a small amount of impurities. The synthetic cocarboxylase gives a yellow color with the formaldehyde-azo-test of Kinnersley and Peters. Thiamin gives a red color.

I wish to thank Merck and Company, and Winthrop Chemical Company, for generous gifts of synthetic vitamin B_1.

10053 P

New Activators of the Carboxylase System and the Function of Cocarboxylase.

HENRY TAUBER.

From the Research Laboratory of the McLeod Infirmary, Florence, South Carolina.

The enzyme carboxylase which plays an important rôle in carbohydrate metabolism of plants was discovered by Neuberg in 1911. Only 21 years later was it shown by Auhagen[1] that the activity of this enzyme depends on the presence of a specific coenzyme. At the same time Auhagen found that for full activity Mg ions are necessary. This was also known to be the case with zymase. Recently Lohmann and Schuster[2] have shown that cocarboxylase is the pyrophosphoric acid ester of thiamin (vitamin B_1 pyrophosphate) and that manganese ions are better activators than magnesium ions. Copper, iron, bismuth, and cadmium salts, as well as sodium fluoride inhibit the activity of the enzyme carboxylase.[3]

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[2] Lohmann, K., and Schuster, P., *Biochem. Z.*, 1937, **294**, 188.

[1] Auhagen, E., *Z. Physiol. Chem.*, 1932, **204**, 149.

Some New Activators. I have tested a series of salts and found that NaC1, Na_2SO_4, KC1, as well as NaCN activate the carboxylase-cocarboxylase system. While Mn and Mg ions, respectively, activate best, the order of activation obtained by NaCN is close to that shown by $MgC1_2$. For example activation by 0.1 mg Mg as $MgC1_2$, is slightly more than by 0.4 mg of Na as NaCN. With 4 mg of Na, of any of the 3 neutral salts, good activation was obtained. Li_2SO_4 and $NaNO_3$ did not activate. The fact that NaCN does not inhibit proves that heavy metals are not involved in this catalysis. NaCN activates, probably, because it forms a more reactive "enol" compound (cyanohydrin) with pyruvic acid. For these salt activation experiments bottom yeast washed alternately with a solution of acid phosphate and alkaline phosphate was employed.[2]

It is believed that cocarboxylase combines with the enzyme carboxylase to form a new compound which in the presence of Mg ions becomes highly active. This compound, however, has not been isolated. Nor has the enzyme carboxylase yet been obtained in pure state. Thus the rôle of cocarboxylase is unknown.

A Specific Function of Cocarboxylase. The following experiments show that one of the functions of cocarboxylase is to protect carboxylase, a very labile enzyme, from destruction. Brewers' bottom yeast was extensively washed with water and dried at room temperature. The dry yeast was then freed of cocarboxylase (by alkaline washing at 30° using a shaking machine)[2] and suspended in 10 volumes of phosphate of pH 6.2.

Experiment 1. One cc of yeast suspension and 30 μg of cocarboxylase* in 1 cc phosphate of pH 6.2 were placed in the main compartment of a Warburg vessel (of 17 cc capacity and 2 side arms). In one side arm 0.5 cc of sodium pyruvate (5 mg of pyruvic acid containing 0.1 mg of magnesium as $MgCl_2$) was placed. The vessel was connected with a Warburg-Barcroft respirometer and shaken for 105 min at 30°. Then the stopcock was closed and after 15 min of further shaking the pyruvate was washed in from the side arm.

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[2] Lohmann, K., and Schuster, P., *Biochem. Z.*, 1937, **294**, 188.

[3] Discussed by Oppenheimer, C., *Die Fermente,* Supplement, 1938, **2**, 1421.

* In all experiments described in this paper synthetic cocarboxylase[4] which had been freed of inorganic salts and was 6 times recrystallized,[5] and natural crystalline (yeast) cocarboxylase were employed with identical results.

[4] Tauber, H., *J. Am. Chem. Soc.*, 1938, **60**, 730.

[5] Tauber, H., PROC. SOC. EXP. BIOL. AND MED., 1938, **38**, 888.

Experiment 2. In another vessel the pyruvate was placed in one side arm and the cocarboxylase was placed in the second side arm while the main compartment of the vessel contained 1 cc of yeast suspension. This vessel also was shaken for 120 min and then the pyruvate and cocarboxylase were added. Cocarboxylase solutions are very stable between pH 4 and 10 at 30°.

Experiment 3. Here 30 μg of synthetic vitamin B_1 (Merck) in 0.1 cc phosphate of pH 6.2 and 1 cc of yeast suspension were placed in the main compartment of the Warburg vessel. The cocarboxylase and pyruvate were added at the end of 120 min.

Experiment 4. The content of this vessel was similar to that of Experiment 1. Cocarboxylase, however, was replaced by 1 cc of H_2O.

It may be seen from Experiment 1, Table I, that when cocarboxylase was immediately added to freshly washed yeast and kept at 30° for 120 min in the presence of air CO_2 formed after the addition of pyruvate very rapidly. If the washed yeast, however, was kept for the same length of time without cocarboxylase the enzyme carboxylase lost almost all of its activity and there was hardly any CO_2 formed (Experiment 2). Thiamin had no protective action on carboxylase and no cocarboxylase was formed during the duration of the experiment from the vitamin (Experiment 3). The yeast suspension without added cocarboxylase did not form CO_2 from sodium pyruvate (Exp. 4). Other experiments (not included in Table I) have shown that the protective function cannot be replaced by Ba-adenosinetriphosphate, Mg-hexosediphosphate, or by reduced glutathione.

TABLE I.

Exp. No.	Content of main compartment during first 120 min.	CO_2* in 10 min.	CO_2* in 20 min.	CO_2* in 40 min.
1	Yeast suspension and cocarboxylase	40	66	102
2	Yeast suspension alone	4	12	24
3	Yeast suspension and thiamin	5	12	24
4	Yeast suspension and water	0	0	0

*After addition of side arm contents.

Cocarboxylase (pyrophosphoric acid ester of thiamin) has a specific protective action upon the enzyme carboxylase.

I am greatly indebted to Professor Lohmann for a sample of natural cocarboxylase, and to J. Ruppert Brewery, through the kindness of Mr. E. Muhlhausen, for furnishing the yeast.

10054 P

Pathologic Changes Produced by Subcutaneous Injection of Rattlesnake (Crotalus) Venom into *Macaca mulatta* Monkeys.*

HERBERT K. FIDLER, RICHARD D. GLASGOW AND EMMETT B. CARMICHAEL.

From the Department of Pathology and the Department of Physiological Chemistry, University of Alabama, Alabama.

Reports of the gross and microscopic lesions in fatal cases of rattlesnake poisoning are rare. Knowledge of the pathologic changes is based mainly upon findings in experimental animals.[1] These changes have been described in detail in dogs.[2,3] Taube and Essex[3] administered large doses of venom (crotalin) intravenously and produced widespread hemorrhagic lesions throughout the body. In nature, however, the venom is usually injected subcutaneously. It is our purpose to report the lesions produced by the subcutaneous injection of a lethal dose of crotalin into the *Macaca mulatta*[4] monkey.

Nine young *Macaca mulatta* monkeys were given rattlesnake *(Crotalus atrox)* venom into the subcutaneous tissues of the lumbar region. The dose, given as a 1% solution in saline, corresponded to 7 to 10 mg of the dried venom per kilo of body weight.

For several hours after injection the animals behaved about as usual, but later became weak, lethargic, and refused to eat. The lethargy and weakness increased in severity until death occurred at an average of 36.5 hr after injection.

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* Aided by a grant from the American Association for the Advancement of Science.

[1] Noguchi, H., *Snake Venoms*, Carnegie Institution of Washington, 1909.

[2] Jackson D., *Southern Med. J.*, 1929, **22**, 605.

[3] Taube, H. N., and Essex, H. E., *Arch. Path.*, 1937, **24**, 43.

[4] Zuckerman, S., and Fulton, J. F., *The Nomenclature of Primates Commonly Used in Laboratory Work*, Tuttle, Morehouse & Taylor Co., New Haven, 1934.

Postmortem examination revealed in the subcutaneous tissues an intense hemorrhagic edema which involved about 50% of the body area. It extended from the mid-thoracic region to the buttocks and groin, and, in several cases, down as far as the knees. At the advancing edges of the lesion there was marked edema but no hemorrhage. The regional lymph nodes in the groin, and to a less extent the iliac, pre-aortic, mediastinal and axillary nodes, were swollen and red. In the heart there were small subendocardial hemorrhages in the left ventricle in the region of the interventricular septum near the aortic ring. This occurred in 6 of the 9 animals. Other lesions were not constant and consisted in one animal of small sub-pleural hemorrhages, and small sub-capsular hemorrhages of the liver, and in 2 others of petechial hemorrhages into the mucosa of the cecum. The lungs and abdominal viscera were pale and dry.

Microscopically, in the region of subcutaneous hemorrhagic edema, necrotic changes were found in the walls of small blood vessels and capillaries. Actual ruptures of the walls were seen in places. Many of the vessels contained thrombi which partially or completely filled the lumens. The surrounding tissues were infiltrated with erythrocytes, fluid, and scattered collections of polymorphonuclears. At the advancing edge of this lesion the blood vessels showed only dilatation and some swelling of the endothelium, while the exudate consisted mainly of fluid, with very few erythrocytes. The regional lymph nodes revealed marked dilatation of the medullary sinuses, but little change in the cortex. The medullary sinuses contained erythrocytes and large phagocytic reticulo-endothelial cells, some of which were distended with as many as 15 to 30 erythrocytes. No necrotic or thrombotic lesions were found in the blood vessels or lymphatics of the lymph nodes. Some polymorphonuclear infiltration and fatty degeneration of muscle were observed in the region of the subendocardial hemorrhages of the heart. The hepatic cells showed slight granular degeneration, and the convoluted and collecting tubules of the kidney showed granular and fatty degeneration. No definite glomerular lesions were present. The lungs, gastrointestinal tract, spleen, pancreas, adrenals, gonads and urinary bladder showed no significant changes.

It is evident from these observations that crotalin acts as a powerful tissue irritant, causing necrosis and actual dissolution of blood vessels. At the edge of the subcutaneous lesion the venom is more dilute, the vascular lesions less severe, and it is from here that maximum absorption of venom probably takes place. The regional lymph nodes show the most intense reaction, but as the venom-containing edematous fluid passes by way of the abdominal and thoracic chains of nodes into the thoracic duct and subclavian vein, lymphadenitis is seen in these other nodes, varying in severity in direct proportion to their proximity to the primary lesion.

Clinical observations and the autopsy findings of widespread vascular dilatation, hemorrhage, and edema led Taube and Essex to conclude that their animals died in shock. Whereas shock seems the most probable major factor in the death of animals subjected to venom intravenously, the evidence is not so clear for this assumption following the subcutaneous administration. Our animals showed none of the generalized vascular lesions which Moon[5] has suggested as the pathologic basis of shock. There was only a subcutaneous loss of blood and fluid, which was so extensive, however, that it might be considered capable of causing shock.

10055 P

Cerebral Blood Flow Changes During Insulin and Metrazol (Pentamethylenetetrazol) Shock.

B. S. LEIBEL AND G. EDWARD HALL. (Introduced by Frederick Banting.)

From the Department of Medical Research, Banting Institute, University of Toronto.

The use of insulin and metrazol in shock therapy for schizophrenia is now well known.

Using a greatly modified, flexible thermoströmuhr simultaneous records of blood flows in carotid artery and meningeal branch of the jugular vein were made in rabbits throughout the course of 128 shock experiments.

Normal venous and arterial blood flows were recorded for one hour. Blood was taken for initial glucose determination.* Insulin was injected subcutaneously (2 units per kilo). Blood sugars were estimated at half-hour intervals for 1½ hours, after which 15-

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[5] Moon, V. H., *Arch. Path.*, 1937, **24**, 642, 794.

* True glucose was determined on 0.3 cc blood precipitated by the method of Herbert and Bourne[1] and glucose determined by a modified Shafer-Hartman reagent (Harding and Downs[2]).

[1] Herbert, F. K., and Bourne, M. C., *Biochem. J.*, 1930, **24**, 299.

[2] Harding, V. J., and Downs, C. E., *Can. Chem. Met.*, 1932, **16**, 12.

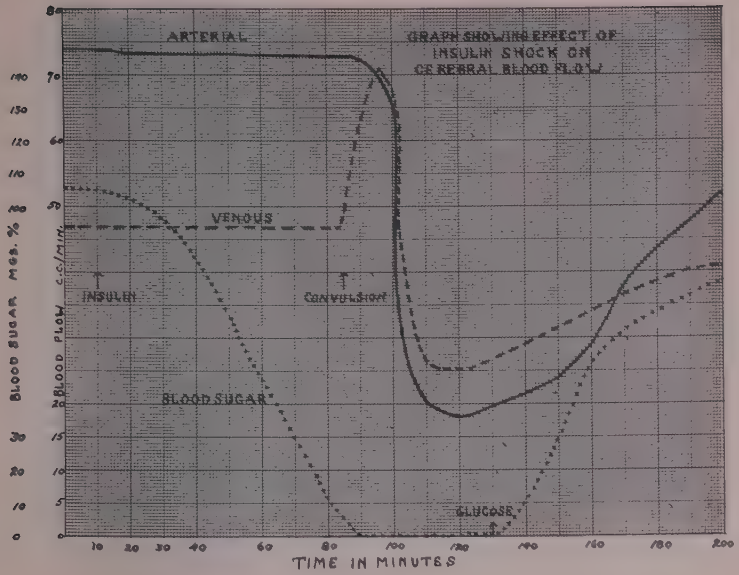

Fig. 1.

minute samples were taken. Blood flow readings were taken at intervals of 2 minutes or less.

The animals usually developed convulsions within 1½-2 hours (Fig. 1). At the onset of the convulsions the blood sugar was always 6 mg % or less. During the convulsions the blood sugar was always undetectable. In the coma stage, following the initial convulsion, the blood sugar fluctuated from 6 mg % to 8 mg %. Spontaneous secondary convulsions frequently occurred, during which time the blood sugar was again undetectable.

At the onset of the primary convulsion the blood flow (Fig. 1) in the cerebral branch of the jugular vein increased very markedly, even up to 50%, while at the same time the arterial blood flow remained constant. This initial phase was followed by a simultaneous decrease in both arterial and venous flow to as low as 25% of the normal level.

In cases where the animals did not go into convulsions there were no significant changes in blood flow, even though the blood sugar had decreased from 95 mg % to 6 mg %.

Shock was induced by metrazol injections in another series of animals prepared in a manner similar to that already described. It has been found that typical convulsions are produced in rabbits (2-3 kilo) by the intravenous injection of 0.75 cc of a 4 % solution of metrazol in distilled water.

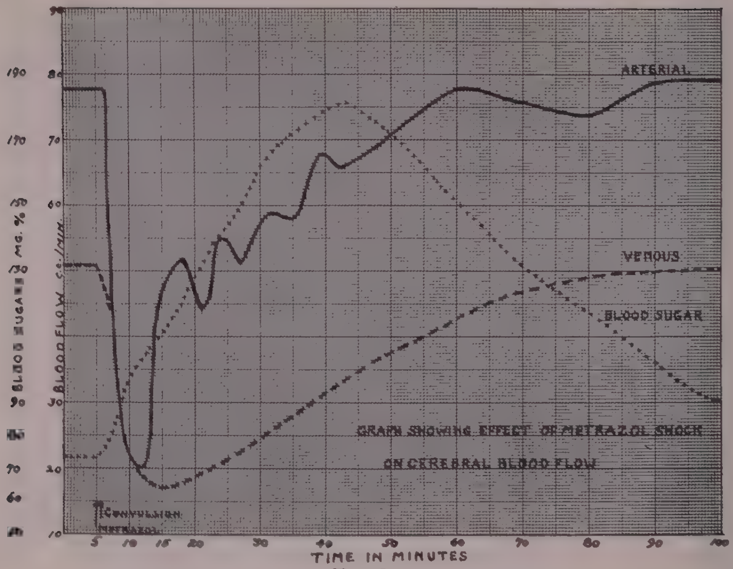

FIG. 2.

Following the injection of an effective dose of metrazol (Fig. 2) the animal almost immediately went into a very typical convulsion which lasted about 2 minutes. This was followed by a semi-comatose state which persisted for several hours. Coincident with the beginning of the convulsive state the arterial and venous blood flows decreased rapidly to one-half or even one-fifth of the normal flows. The blood flow rates returned to normal in an irregular fashion, suggesting the widespread cerebral arteriolar effects brought about by the metrazol injections.

The tremendous fall in blood pressure during the actual convulsion made it impossible to obtain blood samples from the ear for sugar estimations. However, in the post-convulsion state there was a definite hyperglycemia which persisted for several hours. (Fig. 2.)

Thus in insulin shock there was an initial increase in venous return from the brain, suggesting a decrease in size of the brain, while the arterial flow remained constant. This phase was promptly followed by a simultaneous decrease in blood flow in both the arterial and venous sides to such an extent that cerebral anemia was inevitable.

The blood flow changes during metrazol shock were even more rapid. The decrease in cerebral blood flow, both arterial and venous, was sufficient to produce a severe and prolonged cerebral anemia.

10056 P

Action of Pentamethylenetetrazol (Metrazol) and Insulin on Brain Potentials of the Rabbit.

J. E. Goodwin, D. P. C. Lloyd and G. Edward Hall. (Introduced by Frederick Banting.)

From the Department of Medical Research, Banting Institute, University of Toronto.

The use of pentamethylene tetrazol (metrazol) and insulin shock therapy in schizophrenia has prompted several investigations in this laboratory upon the actions of these agents given in convulsive doses. This report deals with some of these actions upon the potentials recorded from the intact rabbit cortex.

Recordings have been made with electrodes of the type described by Rheinberger and Jasper[1] placed symmetrically on the precentral and striate areas of the rabbit. Potentials were led through condenser coupled amplifiers to moving coil oscillographs. Other records were made with paired silver tube electrodes (Adrian)[2] following trephination. Curare and artificial respiration have been

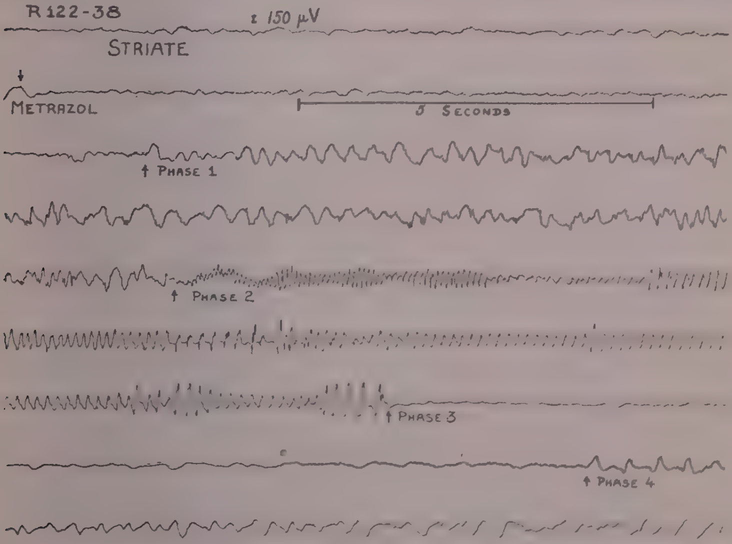

Fig. 1.

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[1] Rheinberger, M. B., and Jasper, H. H., *Am. J. Physiol.*, 1937, **119**, 186.

[2] Adrian, E. D., *J. Physiol.*, 1937, **88**, 127.

used in experiments with metrazol to obviate the gross movement artefacts, but with insulin, the use of curare has been found impractical, due to its hyperglycemic action, which prevented the occurrence of convulsions in all our earlier experiments.

Metrazol. Fig. 1 shows a continuous record of the average metrazol convulsions, the potentials being recorded with paired leads on the area striata. There are 4 clearly defined phases following the latent period, which varies from 3-12 seconds. Phase 1 lasts from 2-21 seconds in primary convulsions and has a wave form very close to that recorded in human petit mal epilepsy. This phase is usually absent in the later secondary convulsions which often occur. Phase 2 lasts from 23-75 seconds, phase 3 from 0-13 seconds, while phase 4 is prolonged, lasting for one hour and up.

The first and second phases are probably due to the direct action of the metrazol. The third phase is possibly a true post-activity depression. The fourth phase may be due to the diminished blood flow noted by Leibel and Hall[3] with a resultant impairment of cortical function. No exact correlation with motor phenomena has as yet been possible due to the necessity for using curare.

Insulin. Fig. 2 shows representative excerpts from a record obtained with bilateral leads on the precentral and striate regions. There is a progressive increase in slow wave activity during the preconvulsive stage. Following convulsion the slow random type of wave continues for some time. Glucose, given in either the preconvulsive or postconvulsive stage, will interrupt the slow wave production, substituting a more normal type of activity. Imme-

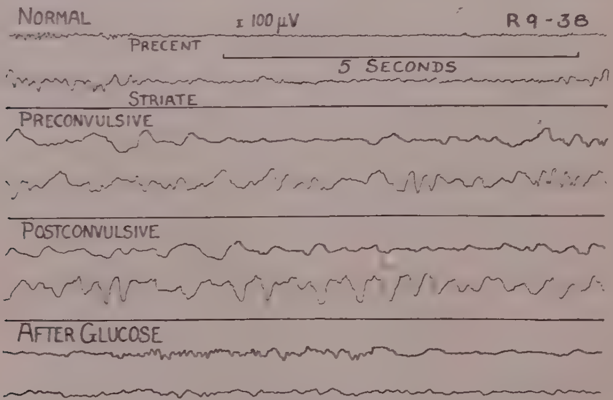

FIG. 2.

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[3] Leibel, B. S., and Hall, G. E., PROC. SOC. EXP. BIOL. AND MED., 1938, **38**, 894.

diately following a convulsion there may be a period of post-activity depression as in the case of the metrazol experiments.

A prolonged course of injections of insulin or metrazol in convulsive doses, (30-36 injections over 58-63 days) has not appreciably altered the course of the convulsive response from that observed in previously untreated animals.

The most conspicuous similarity between the actions of metrazol and insulin on cortical potentials is the prolonged phase of random slow wave production, which may possibly be correlated with the period of reduced cerebal blood flow[3] and coma.

10057

Lead Analyses of Hair as an Indication of Exposure to Lead.

Joseph L. Melnick and George R. Cowgill.

From the Laboratory of Physiological Chemistry, Yale University, New Haven, Conn.

Meillère[1] believed that the hair acts as a channel of lead excretion from the body. The implications of this finding, if correct, in relation to medical and industrial problems involving serious exposure to lead are obvious. Inasmuch as Meillère made no attempt to determine the extent of contamination of his material, his thesis requires further support before it can be accepted. Previous work in this laboratory[2] has confirmed that of numerous investigators indicating that lead is extremely widespread. It is obvious, therefore, that before this question of true lead content of hair can be studied properly, it is necessary first of all to obtain a complete removal of that which is present as an external contaminant. Experiments were performed for the purpose of revealing how such contaminating lead may be removed. For example, after treatment with ether to remove lipoidal material, hair was washed in soap solution, and then in acetic and nitric acids with the idea that the lead could be removed as the soluble salts, but this technic gave unsatisfactory results. Finally, trials were made of the efficacy of treatment with diphenylthiocarbarzone in chloroform, the reagent found so useful in analysis for lead. When lead in known amounts was added to hair to simulate natural conditions of contamination, it was not possible by any of the above methods used to bring the concentration down to what might be regarded as normal levels, such as those characteristic of Group IA in Table II.

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[1] Meillère, M. G., *Compt. rend. Soc. de biol.*, 1902, **54**, 1134.

[2] Horwitt, M. K., and Cowgill, G. R., *J. Pharm. Exp. Ther.*, 1937, **61**, 300.

The problem of metabolic loss of lead through the hair was then approached by means of experiments on rats. The animals, the hair of which had been clipped, subsisted on a low-lead diet for a period of about 3 weeks, during which an amount of hair sufficient for analysis had grown back; at this time the animals were clipped again, and the hair examined for lead. The rats then received lead, in the form of the acetate, both by mouth and by subcutaneous injection, until a new crop of hair appeared, a period again of approximately 3 weeks. In taking hair for the second analyses, care was exercised to avoid the region where the injections were made. The data obtained are presented in Table I. All analyses were made by the Horwitt-Cowgill method.[3]

TABLE I.

Body wt		Amt of lead given by		Amt of lead in hair in relation to lead administration	
Initial g	Final g	Stomach Tube mg	Injection mg	Before p.p.m.*	After p.p.m.*
280	220	60	45	4	14
380	320	80	60	2	18
148	172	50	41	2	7
86	133	32	36	4	14
110	186	41	45	6	11
90	210	0	0	3	25

*parts per million.

The decrease in weights of the adult animals and the relatively poor growths exhibited by the younger rats indicate that the lead was present in toxic amounts. Although the lead content of the hair increased somewhat after lead administration, the fact that the control animal gave a figure slightly higher than any of the experimental rats constitutes ample evidence that lead is not excreted in the hair in this species. The lack of depression of the control animal and its greater activity doubtless account for its greater contamination.

As it is now evident that lead in hair cannot be regarded as that in process of excretion from the body, any found in the hair must represent external contamination. From this it follows that a high content of lead in the hair is sufficient indication that the individual is exposed to high concentrations of lead dust, a form of lead particularly dangerous because it can be inhaled and thus absorbed by the body.[4] To obtain some degree of confirmation of this hypothesis, the hair secured from 30 persons has been analyzed and considered in relation to probable serious exposure to lead. The first group, consisting of 22 persons, includes students, physicians, and individuals from various trades not characterized by undue exposure to lead. The 8 individuals in the second group are paint scrapers, lead smelters, and workers in the scrap metals trade.

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[3] Horwitt, M. K., and Cowgill, G. R., *J. Biol. Chem.*, 1937, **119**, 553.

TABLE II.

		Lead in hair in parts per million	
Group	No. of individuals	Range	Average
I A	20	10- 41	21
B	2	94-140	117
II	8	135-850	510

Two individuals with no apparent undue exposure to lead proved to have considerably greater concentrations of the metal in the hair than others in the "normal" group. They are indicated by the letter B in the table. From the values presented it might be reasoned that these 2 persons should be classified with Group II, but since they were not knowingly exposed to lead, they have been placed in a separate division. The evidence in the table indicates that exposure to lead dust increases the lead "content" of the hair considerably. On the basis of the averages presented in the last column of Table II, this increase over the "normal" is about 25 times.

A practical industrial application of these findings would seem to be in the analysis of the hair of workers at intervals to determine not only the degree of their exposure to lead dust, but the efficiency of the devices used and measures taken to reduce the concentration of lead in the air of the plant. As was pointed out at the beginning of this communication, several experiments failed to reveal any satisfactory method by which contaminating lead may be washed from hair.

Summary. 1. The administration of lead salts to young white rats did not lead to an increased amount of the metal in the hair. Therefore the view that lead is "excreted" through the hair is not supported by the results of this study. 2. On the basis of observations made on 30 human beings with variable exposures to lead it is concluded that the amount of lead to which an individual is exposed may be estimated with reasonable accuracy by analysis of the hair for this metal.

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[4] Aub, J. C., Fairhall, L. T., Minot, A. S., and Reznikoff, P., *Lead Poisoning. Medicine Monographs*, Vol. VII, Baltimore, Williams and Wilkins Co., 1926. See particularly pp. 45-48.

10058

Gonad-Stimulating Abilities of Male and Female Rat Pituitary Glands.*

A. A. HELLBAUM AND R. O. GREEP.

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Functional differences in the hypophysis of male and female rats have been demonstrated through the use of gonadal transplants. Goodman[1] obtained only follicular growth in ovaries transplanted into the anterior chambers of the eyes of adult males, while corresponding implants into female rats resulted in the production of corpora lutea. Pfeiffer[2,3] confirmed and extended the experiments of Goodman in finding that ovarian grafts in the anterior eye chambers of normal males or males whose testes had been removed and reimplanted ectopically, showed only follicular growth. Evans, Simpson and Pencharz[4] found that transplants of fresh pituitary tissue from normal male rats produced a purely follicular response in the ovaries of immature females. On the other hand, Lipschütz and Reyes[5,6] reported that male hypophysis induced luteinization in 100% of the immature female rats, whereas no lutein tissue developed with the use of female hypophysis.

Since the ovarian response to pituitary implants is determined to a considerable extent by active secretion of the gland after implantation, the present report concerns the ovarian stimulation induced

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* Aided in part by a grant from the Research Appropriation of the University of Oklahoma School of Medicine.

[1] Goodman, LeRoy, *Anat. Rec.*, 1934, **59**, 223.

[2] Pfeiffer, C. A., *Am. J. Anat.*, 1936, **58**, 195.

[3] Pfeiffer, C. A., *Anat. Rec.*, 1937, **67**, 159.

[4] Evans, H. M., Simpson, M., and Pencharz, R., PROC. SOC. EXP. BIOL. AND MED., 1935, **32**, 1048.

[5] Lipschütz, A., and Reyes, G., *Compt. rend. Soc. de biol.*, 1932, **109**, 1330.

[6] Lipschütz, A., *Quart. J. Exp. Physiol.*, 1935, **25**, 109.

TABLE I.
Effects of Normal Male and Female Rat Pituitary Glands on Ovarian Response of Normal 21-day-old and Hypophysectomized Immature Rats.

Donors			Test rats				
				Ov. wt and No. of rats showing			
				Follicles only		Corpora lutea	
Age	Sex	No.	Total No. Rats	No. of Rats	Ov. wt Range (mg)	No. of Rats	Ov. wt Range (mg)
Tested on normal immature female rats.							
25 days	M	15	3	3	41-74		
40 ”	M	9	3	3	36-44		
2 mo.	M	6	3	3	42-63		
3 ”	M	16	8	6	33-46	2	52-64
4 ”	M	6	3	3	32-39		
6 ”	M	12	6	3	54-74	3	88-109
12 ”	M	8	4	4	17-53		
18 ”	M	10	5	4	27-71	1	112
25 days	M	14	2			2	106-108
2 mo.	M	12	3			3	76-118
3 ”	M	11	3			3	77-88
6 ”	M	8	2			2	134-152
4-12 mo.	F	49	11			11	17-49
Tested on hypophysectomized immature female rats.							
4-18 ”	M	98	15	14	15-48	1	37
4-18 ”	M	24	2			2	67-83
25 days	M	30	1			1	93

Ovaries of control immature rats weighed an average of 12.5 mg; those of hypophysectomized rats averaged 5 mg.

by the relative amounts of gonadotropic factors present at a given time. Acetone dried powder of rat pituitary glands was injected at different dose levels and the ovarian response determined. The hypophyses of 127 male rats of various ages, as shown in Table I, were tested in 45 female rats 21 days of age, while those from 152 other males were tested on 18 hypophysectomized immature female rats. Similarly the effect of 49 adult female pituitaries was noted in 11 normal 21-day-old recipients.

The ovaries of 31 of the 45 normal rats injected with male rat pituitary powder weighed less than 75 mg and corpora lutea were found in only 2 instances. With the administration of pituitary powder from a greater number of males the ovarian responses exceeded 75 mg and luteinization occurred in every case. Of 18 hypophysectomized test rats injected with male pituitaries, the ovaries of all but 3 showed only follicular stimulation; the 3 hypophysectomized animals whose ovaries contained corpora lutea were injected with a greater number of male pituitaries and the ovaries weighed 67, 83 and 93 mg. It thus seems that the hypophysis of the normal male rat is relatively deficient in, but not free of, the luteinizing factor. The pituitaries of 49 adult females induced luteinization in every instance irrespective of the magnitude of the ovarian enlargement.

Experiments now in progress indicate that castration qualitatively alters the activity of the gonadotropic complex of the hypophysis in rats. A pituitary gland no longer under the influence of the testes shows an increase in the luteinizing factor, which becomes definitely evident about the 20th day after gonadectomy. The concentration of the luteinizing factor becomes such that from the 1st to the 11th month after the operation, injection of pituitary powder from these animals produces heavy luteinization in ovaries of normal and hypophysectomized test rats in every instance. In time, however, the luteinizing capacity is again diminished and the pituitary glands become devoid of this factor even at high dosages.

Conclusions: Assays of male and female rat hypophyses were made by injecting acetone dried glands into normal and hypophysectomized immature female rats. Male hypophyses stimulated follicular development only, unless large amounts of the desiccated glands were administered. The hypophyses of adult female rats induced luteinization at all dose levels.

10059

Respiratory Effects from the Passage of Polarizing Currents Through the Medulla Oblongata.*

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From the Physiology Laboratory, University of Michigan, Ann Arbor.

In a series of experiments now extending over several years we have been attempting to investigate the site and functioning of the central respiratory mechanism by exposing the posterior portion of the floor of the fourth ventricle and studying the effects of local cooling of that region and the effects of the application of certain drugs to it. It was felt that additional information might be gained by studying the effects of the passage of polarizing currents through this part of the brain stem for it seemed likely that if too great current density was avoided, it might be possible in this way to produce temporary depression of function without causing irreparable tissue damage.

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* These experiments were supported in part by a grant from the Rockefeller Foundation to Robert Gesell for studies on respiration.

Twenty experiments have been performed. In all these, dogs anesthetized either with morphine and urethane or with pentobarbital were used. As one electrode we used the instrument which had previously served as our applicator in the experiments on central cooling. The silver chloride plated silver tip of this electrode, triangular in shape, measuring about 3 mm on a side, was placed in light contact with the floor of the fourth ventricle in the calamus scriptorius. A large, indifferent electrode, also silver-silver chloride was wrapped in cotton soaked in saline and placed, in some experiments, in the mouth, in others beneath the skin over the occiput.

With the electrodes so placed, a current of only a few milliamperes resulted in profound changes in respiration. In some cases the effects were completely reversible while in other cases recovery did not occur, suggesting that permanent damage had been done. Inspection of the brain in these latter cases frequently revealed gross tissue destruction.

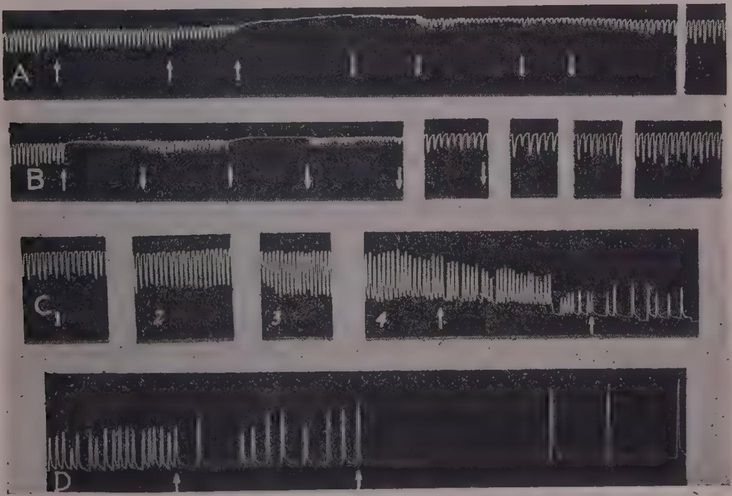

FIG. 1.

Effect of polarizing currents on respiration. Records are of circumference changes of the abdomen, downstroke representing inspiration. Upright arrows indicate increases in current strength, inverted arrows decreases. In records A and B the last arrow indicates decrease of the current to zero. In record C the current was increased in the intervals shown by breaks in the record. In record A the electrode in contact with the surface of the brain was the cathode; in record B, the anode; in record C, the anode; and in record D, the cathode.

The results, somewhat variable, seem to fall into 2 groups. In the majority of the experiments, the effect of the passage of such a current was a decrease in amplitude of respiration proportional to the current strength and independent of the direction of current flow. When respiration ceased, it was with the chest in the expiratory position. These results we believe represent the effect of depression of the entire respiratory center—inspiratory as well as expiratory parts. Two examples of this type of response are illustrated in Fig. 1 A and B.

The other type of response, illustrated in Fig. 1 C and D, is characterized by a marked increase in the duration of inspiration resulting in so-called apneustic respiration. This type of breathing we have also produced by local cooling of the floor of the fourth ventricle[1] and by the application of cocaine[2] and is the result, we believe of depression of the expiratory portion of the central respiratory mechanism leaving the inspiratory portion relatively unaffected. It is interesting that all of the stages between normal respiration and apneusis which have been observed during gradual cooling and the gradual absorption of cocaine may also be seen during the passage of a polarizing current. This is well illustrated in Fig. 1 C. The first effect seen in C_2 is a slight decrease in the duration of the expiratory pause with a small increase in the respiratory rate. In C_3 we see that the expiratory pause is now almost completely abolished with as yet no evidence of inspiratory prolongation, resulting in a considerable acceleration of respiration. In C_4 the expiratory pause has completely disappeared and an inspiratory pause appears and becomes progressively more marked until the respiration is decidedly apneustic in type with the rate far below normal. Complete recovery did not occur in this case, the respiration remaining somewhat apneustic in type after cessation of the current. Re-application of the current, now in the opposite direction, Fig. 1 D, greatly intensified the apneusis.

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[1] Nicholson, H. C., *Am. J. Physiol.*, 1936, **115**, 402.

[2] Nicholson, H. C., and Sobin, S., in press.

10060 P

Spherule Formation and Endosporulation of the Fungus Coccidioides *in vitro*.*

Arthur R. Lack. (Introduced by Ernest C. Dickson.)

From the Department of Public Health and Preventive Medicine, Stanford University School of Medicine, San Francisco.

Since the causative agent of coccidioidal granuloma (*Coccidioides immitis* Stiles) was demonstrated to be a fungus and not a protozoon by Ophüls and Moffitt,[1] the reason for the formation of spherules in the animal body has evaded explanation. MacNeal and Taylor[2] were the first to attempt to obtain growth of spherules outside of the animal body. They inoculated pus containing spherules into tubes of ascitic fluid and gelatinized horse-serum to which sterile tissue had been added and cultured them anaërobically. They observed that parasitic forms, *i. e.*, spherules, continue to multiply for a time by endogenous formation. Takahashi[3] repeated the experiments of MacNeal and Taylor, but was unable to observe the formation of endosporulating spherules. Ciferri and Redaelli,[4] however, were able to confirm the continued formation of endosporulating spherules from pus containing non-endosporulating spherules. On the other hand, their anaërobic tubes inoculated with fungus from normal cultures showed nothing interesting.

In an effort to find the prerequisite conditions for spherule-formation in the case of coccidioides, a series of semi-anaërobic experiments was performed.

Procedure: A semi-anaërobic constricted tube of the Hall[5] type was used. A series of tubes was filled well above the point of constriction with filtered glucose-broth having a pH of 7.2 and heated to 100°C for 15 min. When cooled to room temperature, they were ready for inoculation. The inoculum was prepared by extracting the albumin from a fresh egg and suspending in it a preparation of chlamydospores from an 8 weeks' old fungus culture (from the Stanford Fungus Series, strain No. 46). This inoculum was pipetted into the bottom of the constricted tubes and immediately a sterile marble large enough to close off the constricted neck was dropped in place. By gentle warming partial coagulation of the egg albumin within the lower chamber was obtained, the heat being insufficient either to kill the organism or completely coagulate the egg albumin. Four control tubes were prepared: one as described above but uncoagulated by heat, one containing the inoculum with no marble, one with fresh egg albumin and one with the suspension of chlamydospores without egg albumin. The tubes were then incubated at 37°C. Samples were taken every 24 hr and examined by coverslip and stained smear preparations.

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* This study is being aided by the Rosenberg Foundation, San Francisco.

[1] Ophüls, W., and Moffitt, H. C., *Phil. Med. J.*, 1900, **5**, 1471.

[2] MacNeal, W. J., and Taylor, R. M., *J. Med. Research*, 1914, **30**, 261.

[3] Takahashi, S., *Arch. f. Dermat. u. Syph.*, 1933, **168**, 597.

[4] Ciferri, R., and Redaelli, P., *Soc. internaz. di microbiol., Boll. d. sez. ital.*, 1934, **6**, 141.

[5] Hall, I. C., *J. Inf. Dis.*, 1921, **29**, 317.

Results: At 24 hr, the experimental tubes show a slight enlargement of the chlamydospores within the egg albumin. A rounding-out of previously oval shaped chlamydospores takes place. There is no development of mycelial buds (hyphæ) from either the chlamydospores or the broken mycelial fragments contained in the original chlamydospore suspension. The controls show no changes except for beginning hyphæ formation in the tube containing the fungus without the egg albumin.

By 48 hr, the growth or enlargement of the chlamydospores in the experimental tubes continues with the formation of now small, spherule-like bodies, round in contour and possessing a distinct, greenish capsule. Round bodies are seen which are 20-30μ in diam, are finely granular in structure and possess a greenish, granular capsule. These may be designated the "granular" type of spherule. In these same cultures are found larger round organisms (30-40μ in diam), granular in structure and possessing a thick yellow-green capsule, from which peripherally radiate numerous large spicules. These spicules are quite distinct in structure, being broad at their base and quickly narrowing to a very sharp point; they are analogous to the prickle-forms described by Rixford and Gilchrist.[6] Again the controls show no changes except for growth of mycelium in the tube containing the fungus alone.

After 7 days, coverslip-examinations of portions of the egg albumin of the experimental tubes show many greenish, granular spherules, some of which have the sharp, broad spicules. There are a few clear (vacuolated) spherules possessing wide, refractile, double-contoured capsules which are yellowish green in color. A large spherule of approximately 50μ in diam, with a greenish capsule of medium width, is seen. Within this organism are round or irregularly shaped bodies about 6-10μ in diam. These are endobodies or endospores. The control tubes show no spherule-development; mycelial growth is obtained in the tube with no marble and in the upper portion of the tube with coccidioides alone.

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[6] Rixford, E., and Gilchrist, T. C., *Johns Hopkins Hosp. Reports*, 1896, **1**, 209.

The formation of endospores in the spherules obtained in this series establishes the identity of the spherule-form. It also indicates the maturity of the organism and renders the *in vitro* development of the fungus under certain conditions comparable to the *in vivo* development of the same fungus.

Summary: The parasitic cycle of the fungus coccidioides was reproduced *in vitro*. Spherule-formation with endosporulation was experimentally produced under certain conditions, including partial anaërobiosis and the presence of partially coagulated egg albumin. An interesting type of spherule-formation (granular spherule) and a distinct spicule formation are described.

10061

Influence of Ketene on the Potency of Antipneumococcus Serum.*†

Joseph T. Tamura and M. John Boyd. (Introduced by S. Tashiro.)

From the Departments of Bacteriology and Biochemistry, College of Medicine, University of Cincinnati.

Recently we[1] showed that the treatment of anti-Brucella horse serum with ketene for 35 min or more prevents anaphylactic shock in animals sensitized to the original antiserum. Furthermore, such a serum still retains the major part of its agglutinating antibody. The purpose of this paper is to record the influence of ketene on the protective power of antipneumococcus serum.

Type I antipneumococcus horse serum, containing 200 units per cc (1642-1, Eli Lilly and Co.) was tested. To 10 cc of the serum to be ketenized, 0.6 g of $NaHCO_3$ was added to buffer the acetic acid which is formed during the process. A drop of caprylic alcohol was introduced to prevent foaming. Ketene gas was passed through the serum for 35 min at the rate of 100 bubbles per min (diam of inlet tube, 4 mm). At the end of the ketenizing process the pH of the serum was 7.8. Both the ketene treated and the original antisera were diluted with 0.85% physiological salt solution until 1 unit was contained in 0.5 cc. In this test a constant amount of serum and varying amounts of culture were used.

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* This research was aided in part by a grant from the Craig Yeiser Fund, College of Medicine, University of Cincinnati.

† The authors wish to acknowledge the courtesy of Dr. Mary B. Kirkbride of the New York State Department of Health, and Dr. H. M. Powell of the Eli Lilly and Company.

[1] Boyd, M. J., and Tamura, J. T., Proc. Soc. Exp. Biol. and Med., 1938, **38**, 184.

A New York State Laboratory strain, pneumococcus type I "N" (Neufeld) bacterial collection No. 1 was employed. The first blood-broth transfer of a heart-blood culture from a mouse dying within 48 hr after intraperitoneal inoculation of 0.00000001 cc of culture was used in the test. Organisms were cultured in double infusion blood-broth for 16 hr at 37°C. All dilutions were made in broth so that the desired amount of culture was contained in 1.0 cc.

On the assumption that each pneumococcus colony represents the growth from one organism, the number of pneumococci per cubic centimeter was determined by triplicate blood agar pour plates at the time of the test by the use of 1.0 cc each of 10^{-7}, 10^{-8}, and 10^{-9} dilutions of the culture; the colonies were counted after 48 hr incubation.

All dilutions of cultures were made and all mice were injected within 1 hr from the time the dilutions of cultures were prepared. The diluted cultures containing varying amounts of organisms, were thoroughly mixed with diluted serum and 1.0 cc of the mixture immediately injected intraperitoneally into mice weighing from 18-22 g. Each cc of the mixture contained 1 unit of antiserum.

TABLE I.
Influence of Ketene on the Protective Antibody of Antipneumococcus Serum.

Serum	No. of mice used	Unit of serum used (0.5 cc)	cc of culture used	No. of organisms injected	Results
Ketenized 35 min.	9	1	0.5×10^{-3}	4500000	All protected
	9	1	0.2×10^{-3}	1800000	" "
	9	1	0.05×10^{-3}	450000	" "
Original serum	5	1	0.5×10^{-3}	4500000	Four protected. One died in 75 hr
	5	1	0.2×10^{-3}	1800000	Four survived. One died in 85 hr
	5	1	0.05×10^{-3}	450000	All protected
Control	3	0	1.0×10^{-9}	9	All died within 48 hr
	3	0	1.0×10^{-8}	90	" " " 24-30 hr
	3	0	1.0×10^{-7}	900	" " " 16-20 "

Mice surviving for 96 hours or more were considered protected by the serum. The results of this test are given in Table I.

Twenty-seven mice were protected when injected with a mixture of one unit, or 0.005 cc of ketenized serum and pneumococci numbering from 450,000 to 4,500,000, whereas out of 15 mice injected with one unit of original antiserum and a similar number of organisms one died in 75 hours, another in 85 hours. Nine unprotected control mice died within 16 to 48 hours after injection with from 9 to 900 organisms.

The results of the present study indicate that the protective antibody of antipneumococcus horse serum is unchanged when treated with ketene, and strongly suggest that the ketenization of antisera might be very useful in serum therapy.

10062

Delayed Sedimentation in Antihemocyanin-Systems.

SANFORD B. HOOKER.
From the Evans Memorial, Massachusetts Memorial Hospitals, Boston.

In the course of some turbidimetric observations on precipitative systems, using the Evelyn photoelectric photometer, it became desirable to correlate the measurements with particulation and evenness of sedimentation as determined by direct inspection. Within a certain limited range of rather strong mixtures of *Limulus* hemocyanin and its antiserum it was found that sedimentation was enormously delayed, sometimes requiring several days for completion. The same kind of behavior was noted with the hemocyanin of *Fulgur carica* but not with crude horse serum and its comparatively weak antiserum. If the mixtures were agitated by convective currents, when the tubes were partly immersed in a waterbath, no zone of delayed sedimentation was observed. In weaker mixtures, even though they covered the same range of optimal flocculation, sedimentation proceeded also in the regular way.

One-milliliter volumes of mixtures of a constant amount of antiserum with decreasing quantities of antigen were pipetted into small tubes, 67 x 4.8 mm inside, which were arranged perpendicularly, protected from jar and agitation as far as possible, and kept at room-temperature (*ca* 23°C). In those tubes in which sedimentation was markedly delayed, particulation first occurred in the topmost part of the column of fluid and gradually progressed downward.* Other mixtures particulated, if at all, evenly throughout the column and sedimentation occurred quickly without formation of the sharp upper boundary that characterized the other slowly settling aggregates.

This boundary was usually as clearly marked as that seen in the sedimentation of unagglutinated erythrocytes. The top layer of the settling aggregate gradually thickened and became more opaque while the finely web-like structure of the underlying column remained unchanged in density until the whole aggregate was deposited in the bottom of the tube. In many tubes a clot-like retraction of the aggregated structure occurred; narrow clear zones appeared along the sides of the tubes from which the aggregates had shrunk entirely or to which they remained attached at one or more points. Presumably these points of adhesion were rough or dirty spots on the glass. Occasionally the aggregated structure appeared to cling to a bubble on the surface or showed a funnel-shaped depression in the top. These irregularly occurring adhesions naturally made the sedimentative rates less orderly.

As an example of this peculiar delay in sedimentation, the reaction of *Fulgur* hemocyanin with its antiserum is given in Table I which shows the relation of the speed of settling to the composition of individual mixtures.

In numerous experiments the zone of slowest sedimentation has been rather near but always to the left of the tubes showing most rapid particulation. When the excess of antigen was considerable, although particulation was slow and incomplete, sedimentation occurred without the development of the columnar gel-structure under a clear supernate. With excessive antibody sedimentation was prompt. When the concentrations shown in the table were halved this type of delayed sedimentation was not observed. When the concentrations were doubled, more than a week was required for complete settling in some tubes and the range of the reaction was broadened. The phenomenon did not recur if the final deposits were thoroughly dispersed with a fine capillary pipette and again allowed to flock out.

The behavior of *Limulus* hemocyanin was more irregular. In some ranges of concentration two prominent peaks of delayed sedimentation were seen with a trough of more rapid settling between them. This may plausibly be ascribed to the influence of mixed antigen-antibody systems—*Limulus* blood contains several molecular species differing in size and antigenic specificity.[1]

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* Because these antigens are respiratory proteins it seemed conceivable that the presumably higher oxygen-tension at the top of the column might lead to earlier particulation. However, the use of freshly boiled salt solution as diluent and freshly cooled paraffin oil to overlayer the mixtures thickly did not alter the result.

The general appearance of this phenomenon closely resembles that diagrammed by Basset *et al.*[4] in a recent report on the effect of high pressure upon specific flocculation of the tobacco-mosaic viral pro-

TABLE I.
Reactions of *Fulgur carica* hemocyanin with its antiserum.

Antigen-N, γ*	31	24.8	21.7	12.4	8.7	7.4	5.3	4.4
Time of first particulation, min.	>180	?	41	25	21	21	23	28.5
Depth (mm) of supernate†								
after 1 hr‡		0.5	0.5	1	1.5	2	4s	s
1.5 ”		1.5	1.5	2	3.5	8	32s	S
2 ”		2	2	4	14	24	S	
3 ”		4.5	4.5	18.5	30	35.5		
3.5 ”	s	5	6	23.5	33.5	38		
4.5 ”	s	5	10	29	36	S		
6.5 ”	s	8	23	34	39			
29 ”	S	18	S	S	S			
In supernate of ten-fold stronger mixtures:								
Antigen, γ§	52	20	2	0	0	0	0	0
Antibody‖	0	0	0	0	0	0	0	Trace
Antibody-N in precipitate, γ	811	1110	1071¶	951	853	806	685	636
Ratio Ab N/Ag N**	3.14	4.9	5.8	7.7	9.8	10.9	12.9	14.4

*Contained in 0.5 ml and mixed with 0.5 ml of antiserum 846_3 diluted 1:10.

†The distance of the upper boundary of the settling aggregate from the meniscus.

‡That is, 1 hr after particulation first occurred in the series.

§Estimated by speed of particulation with a standardized serum.[3]

‖Tested with 1.5 γ of antigen-N.

¶This mixture contained 186 γ of antigen, not 217.

s = sedimenting with poorly defined boundary or none.

S = completely sedimented.

** These ratios are surprisingly higher than were anticipated from results with the hemocyanin of the closely related *Fulgur canaliculatus*.[1] An explanation was sought in the possibility that the *carica* preparation, stored in glass for 2 years, might have dissociated into smaller components and thus offer a larger surface with which more antibody could combine. Components about the size of the molecule of hemoglobin would be expected, judging from the magnitude of the observed ratios.[2] However, no specifically reactive material in the diluted *carica* hemocyanin could be made to pass through a membrane with an APD of 14 mμ, which did permit the passage of hemoglobin.

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[1] Hooker, S. B., and Boyd, W. C., *J. Immunol.*, 1936, **30**, 33.

[2] Boyd, W. C., and Hooker, S. B., *J. Gen. Physiol.*, 1934, **17**, 341.

[3] Hooker, S. B., and Boyd, W. C., *J. Gen. Physiol.*, 1935, **19**, 373.

[4] Basset, J., Gratia, A., Macheboeuf, M., and Manil, P., PROC. SOC. EXP. BIOL. AND MED., 1938, **38**, 248.

tein. Inasmuch as this protein and these hemocyanins are of huge molecular size it may be that the formation of slowly settling aggregates is a peculiarity of macromolecular systems. However, the sera I have used were unusually powerful (846_3 contained over 14 mg of antibody-protein per ml) and other sizes of antigenic molecules must be investigated under comparable conditions before such an assumption would be justified.

Summary. An unusual zone of greatly delayed sedimentation was observed in titrations of the hemocyanins of *Limulus polyphemus* and *Fulgur carica* with their precipitins. The extent of the zone was influenced by both the relative and the absolute concentration of the two specific reactants.

10063

Spontaneous Agranulocytosis in the Cat.*

John S. Lawrence and Jerome T. Syverton. (Introduced by W. S. McCann.)

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During the course of making some routine blood counts on cats one animal was found with only 350 white blood cells per mm^3 and no neutrophils in the peripheral blood. A portion of the liver of this animal was ground up with Alundum in Locke's solution. After settling, the supernatant fluid was used for injection into 5 additional cats. This resulted in the development of the same blood picture in 2 of these 5 cats. Following this a similar preparation was made from the liver of Cat 36 and 6 additional cats injected with this material, part of them receiving the material intraperitoneally and a part subcutaneously. This process was continued as shown in Chart 1 until 13 transmissions were accomplished.

Following injection a typical animal has a period of about 5 days when it appears normal. Then, it is frequently noted that food is left in the cage and the temperature is usually found to be elevated (39° to 41°C by rectum). The animal becomes slightly listless and, after a day or two (*i. e.*, the 6th to the 8th day) may not care to stand on its feet. There is no nasal or eye discharge except in a rare

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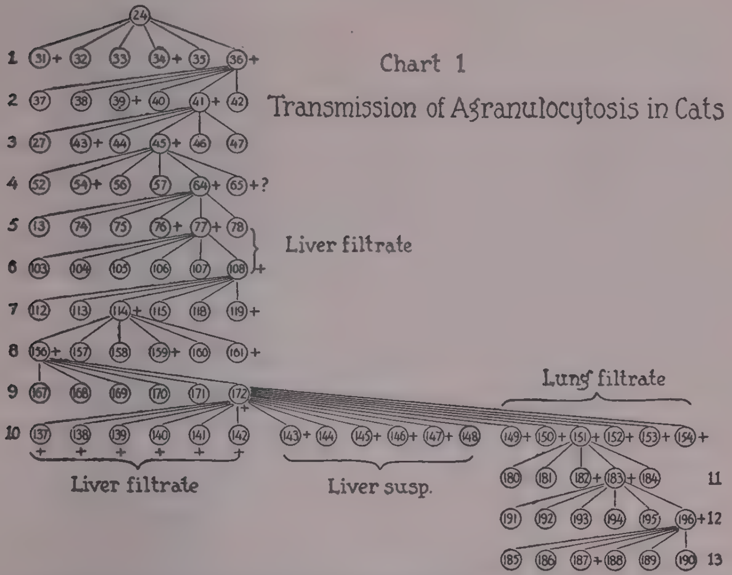
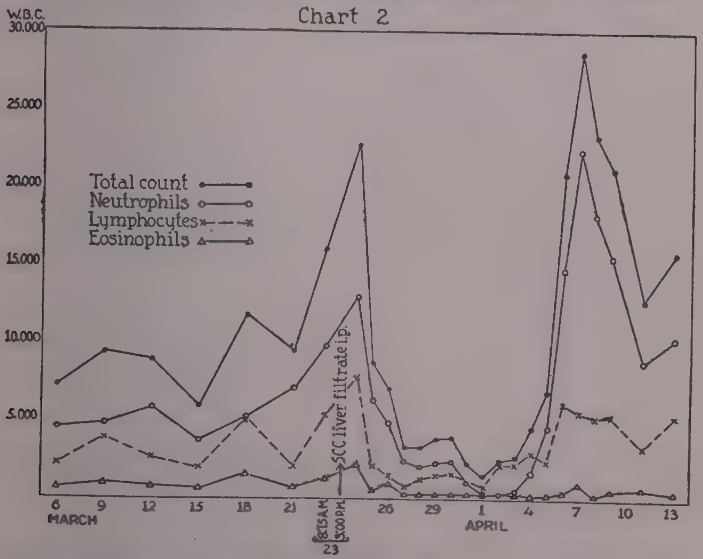
* Aided in part by a grant from the Committee on Scientific Research of the American Medical Association.



Numbers on sides of chart designate successive transfers of the disease. The + marks indicate those animals that developed typical signs of the disease.

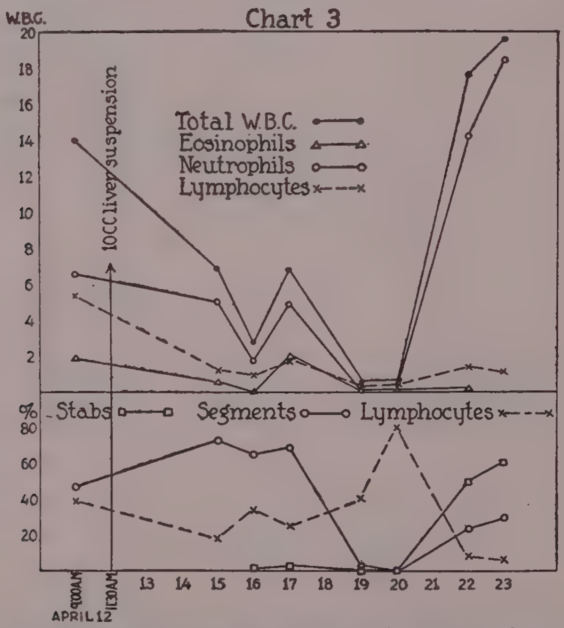


Represents changes in total number of white blood cells in a typical animal following injection of material containing the active agent.

animal. Diarrhea may occur but is, by no means, constant. Coinciding with the pyrexia, anorexia and listlessness there is marked neutropenia and frequently the total white blood cell count goes to a very low level.

Chart 2 represents typical findings as regards the white blood cells in one of the animals that recovered. However, the response is not identical in all animals. In some instances, there is a steady decline until the low point is reached on the 6th to 8th day. In others, there is no change in the level of the white blood cell count until the 6th to 8th day when there is an abrupt fall. In these latter animals, there may be a very high percentage of neutrophils on the day preceding the development of the leucopenia.

The striking thing about the differential formula is that very large numbers of "stab" cells may appear just prior to the leucopenia or, if recovery occurs, at the time when the white blood cell count is increasing. This is well illustrated by Chart 3. The red blood cell count shows no constant variations. The platelets remain normal or increased. After one or 2 days, at the height of the disease, the animal either dies or begins to eat and has a gradual or sudden increase in white blood cells.



Changes in differential count as well as those in total number of the white blood cells are shown. Numbers on upper left side refer to white blood cells in thousands per mm^3.

The number of animals that would recover is not known as animals were sacrificed frequently at the height of the disease in order to get material for study or for transfer purposes. However, 7 animals did recover. In these animals, neutrophil counts below 2000 per mm^3 were maintained from 2 to 5 days, the majority having marked neutropenia for 4 to 5 days.

Thus far, only a few experiments have been carried out that bear on the infectious nature of the disease.

Bacteriological studies were essentially negative. The blood and liver, which provided source material for the inoculation of a wide variety of special media that were incubated under aerobic, anaerobic, and microaerophilic conditions, proved to be bacteria-free in 3 cats and yielded a few extraneous contaminants in 2 additional cats.

Further experiments, which were designed to bear on the filterability, or possible virus nature, of the causative agent, yielded evidence which suggests that a transmissible filterable agent is responsible for the disease. Three groups of cats, Groups A, B, and C, with 6 animals in each, were maintained as entirely separate units under rigid isolation for 18 days. Rectal temperatures were obtained at one to 2-day intervals during this time. Also, white blood cell and differential counts were made at one to 3-day intervals. Following the period of isolation, all of the cats were inoculated with material derived from a single moribund animal, Cat 172, which represented the tenth consecutive passage in the transmission series. The inoculum that was used for 2 groups of the cats, Group A and Group B, consisted in the supernate, unfiltered and as a bacteria-free Berkefeld V filtrate, respectively, of a centrifuged 10% liver tissue suspension that had been prepared by trituration of the liver with Alundum, using Locke's solution as the diluent. The third group of animals, Group C, received a Berkefeld V filtrate derived from the tissues and secretions of the upper respiratory tract. This was prepared as follows: The secretions and mucosa of the upper respiratory tract, including the turbinates and bronchi, were subjected to shaking with glass beads for one hour. The fluid portion then was pipetted off, mixed in equal parts with a 10% suspension of lung tissue, and the resultant mixture centrifuged for one hour at 2500 r.p.m. The supernate, following centrifugation, was filtered through a Berkefeld V filter and the resultant filtrate, which was proven to be free of bacteria, was used as the inoculum for the animals in Group C.

Daily temperature and white blood cell determinations were made following injection. Sixteen of the 18 animals developed typical findings of the disease.

No detailed observations on the pathology of these animals have been made but studies are going on in this connection at present.

Arrangements are being made for the study of other virus diseases in the cat in order to determine whether the agent responsible for this blood picture is a hitherto unknown one or is one that has been known to produce disease in the cat but the effect of which on the blood was unknown.

In conclusion, evidence has been presented which seems to justify the statement that there is a transmissible disease in the cat characterized by neutropenia and leucopenia. The causative agent would seem to be a filterable agent or a virus.

10064 P

Relation of Chemically Induced Activity in Nerve to Changes in Demarcation Potential.*

T. SJÖSTRAND,† F. BRINK AND D. W. BRONK.

From the Eldridge Reeves Johnson Foundation and the Institute of Neurology, University of Pennsylvania, Philadelphia.

Repetitive activity often develops in mammalian nerves as a result of a localized injury (Adrian).[1] The phenomenon has been explained by assuming that circulating currents set up between the depolarized region of injury and the normal nerve result in periodic stimulation, at a frequency which depends on the rate of recovery of the normal nerve surface and the strength of the circulating currents. Recently, Fessard[2] has shown that repetitive activity can result when crystals of certain salts are applied to a nerve trunk, and that the region thus treated may be either positive or negative to the untreated parts. In recent work we have made an extended investigation of chemical activation of nerve, using isotonic solutions containing various effective salts applied to single active fibers dissected from the sciatic nerve of the frog. In these experiments the impulses were recorded at the treated region. This is an ideal preparation for studying the relation between chemically induced activity and changes in demarcation potential.

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* Supported by a grant from the Supreme Council, Scottish Rite Masons.

† Fellow of the Rockefeller Foundation.

[1] Adrian, E. D., *Proc. Roy. Soc. B*, 1930, **106**, 596.

[2] Fessard, A., *L'Activité Rythmique des Nerfs Isolés*, Hermann and Co., Paris, 1936.

The present experiments were undertaken (1) to observe the impulse discharge and the change in demarcation potential by simultaneous measurement of the two through the same pair of matched electrodes, (2) to measure the effect of anions on the demarcation potential of a single fiber, since this has never been observed in studies on the sheathed frog nerve (Wilbrandt,[3] for discussion) and (3) to test our hypothesis that chemically induced activity results only during the period of active exchange of ions in a nerve, and that the degree of activity depends upon the rate of this exchange.

We have observed that activity can occur with a positive change in demarcation potential, as in isotonic NaSCN; or with a negative change in demarcation potential, as when NaCl + KCl (4 times normal) is applied to a fiber previously soaked in NaCl. From this it would seem that a sufficient change in electrical potential, of either sign can disturb the molecular organization sufficiently to cause impulses to develop. However, we have also found activity with no change in demarcation potential (within ±0.1 millivolt), in Na_3Ci. Although these latter changes in demarcation potential were somewhat variable, Fig. 1 shows a clear-cut case of activity occurring when no change in demarcation potential resulted. This

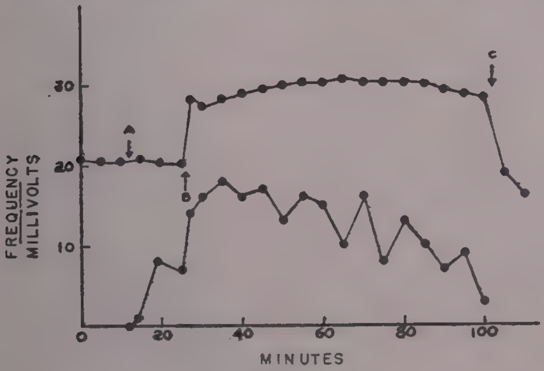

Fig. 1.

Relation between demarcation potential and frequency of response (impulses per second). At A, isotonic Na_3Ci was applied; at B, isotonic NaSCN; and at C, isotonic Na_3Ci. The upper curve is the demarcation potential relative to a region of the fiber depolarized by KCl. The lower curve is the impulse frequency.

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[3] Wilbrandt, W., *J. Gen. Physiol.*, 1937, **20**, 519.

may mean Na_3Ci, by removing Ca^{++}, unstabilizes the molecular organization whose cycle of alteration constitutes the spike potential, while NaSCN or KCl produce their effect by the potential changes set up across this surface layer of molecules. This possibility is being further investigated.

Isotonic NaSCN always stimulated (after preliminary treatment with Na_3Ci or NaCl to remove some of the Ca^{++}), at a time when the demarcation potential went positive compared to that in NaCl. This effect is shown in Fig. 1 and can be consistently obtained provided the nerve be treated previously with a calcium-removing agent. A nerve treated with NaSCN without previous soaking in NaCl or Na_3Ci shows a positive shift of potential of lesser degree, which may be due to the lowering of the K^+ concentration relative to that in Ringer's fluid.

In our previous work we have found a similarity of the cycle of activity in NaSCN to that in KCl when these are applied after lowering the Ca^{++} content of the nerve. The activity goes more or less abruptly to its maximum frequency and then falls off to zero over a period of 5-10 minutes. This transient burst of activity is accompanied by a sudden but persistent change in demarcation potential, in a positive sense for NaSCN, and, as was to be expected, in a negative sense for KCl.

The period of most rapid change in demarcation potential following the application of NaSCN (Fig. 1) is probably associated with the most rapid exchange of anions. Since at this time the activity also shows an abrupt increase in frequency with a slower increase afterward there appears to be a correlation between change in activity and change in interface potential. However, the irregular decline of frequency occurs during the steady persistence of the increased demarcation potential, and the maximum of frequency does not coincide with the maximum rate of change of demarcation potential.

Our hypothesis that chemical stimulation occurs only during the actual exchange of ions is thus supported by these observations. However, the additional hypothesis that the highest frequency of impulses occurs during the most rapid exchange of ions is not confirmed because the maximum frequency does not coincide with the maximum rate of change of demarcation potential. The implications of this latter relationship must, however, be investigated more extensively.

10065 P

Persistent Discharge from Sympathetic Ganglion Cells Following Preganglionic Stimulation.*

M. G. Larrabee and D. W. Bronk.

From the Eldridge Reeves Johnson Foundation and Institute of Neurology, University of Pennsylvania.

If volleys of impulses enter a sympathetic ganglion at frequencies not exceeding 15 or 20 per second, each impulse arriving at a given synapse causes the discharge of not more than one impulse from the corresponding ganglion cell.[1] Thus no repetitive discharge has been observed, and there is evidence that this is also true in certain cases of synaptic transmission in the central nervous system. To account for this it has been assumed that there is a rapid decay of the excitatory state developed by the presynaptic impulses. The neurohumoral hypothesis takes cognizance of this fact by assuming that the acetylcholine is rapidly hydrolyzed.

We have recently discovered conditions in which the statements made in the preceding paragraph do not correctly describe the course of the excitation at the synapses. For we have observed a long-lasting discharge of impulses from ganglion cells, following a brief period of preganglionic stimulation at a frequency of 40 or more per second.

All of the nerves leading to and from the stellate ganglion of the cat were sectioned. The preganglionic trunk was then stimulated while recording the impulses in the postganglionic inferior cardiac nerve.

At the lower frequencies each preganglionic volley initiates a single volley of impulses in the postganglionic nerve. If the frequency of stimulation be 40 or more per second the synchronized discharge of impulses does not continue throughout the course of a period of stimulation but soon becomes random due to the asynchronous activity of the several ganglion cells.[2] When the stimulation of the preganglionic nerve is stopped the discharge from the ganglion does not cease but the random discharge from many cells continues for some time thereafter.

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* Aided by a grant from the Supreme Council, Scottish Rite Masons.

[1] Bronk, D. W., and Pumphrey, R. J., Proc. Soc. Exp. Biol. and Med., 1935, **32**, 1661.

[2] Bronk, D. W., Tower, S. S., Solandt, D. Y., and Larrabee, M. G., *Am. J. Physiol.*, 1938, **122**, 1.

During the high frequency stimulation the number of ganglion cells discharging gradually gets less. At the end of the period of stimulation there is not a sudden, further decrease in activity, but only gradually and over a period of 0.2 to 0.5 seconds do the cells go out of action. Then for the short space of 0.2 to 1.0 second there is no discharge, following which a certain amount of activity again develops and continues for 15 or more seconds. The intervening period of inactivity is apparently related to the phase of decreased irritability revealed by the positive after potential following the period of intense activity.

It is not possible to say what proportion of the ganglion cells participate in this after-discharge or at what frequency, until we have many records of the activity of small groups of cells. For only thus can individual impulses be observed and quantitative measurements made. At the present time we can estimate that in the initial phase the number of cells in action may well be 25% of those responding at the beginning of the period of stimulation. Several seconds after the end of the stimulus the activity falls to 5 or 10%.

How many units continue in action is probably of less significance than the fact that certain of them do continue to discharge for 15 or more seconds after the end of the trains of impulses which developed the excitatory state at the synapse. We are, therefore, forced to conclude that the altered state of the nerve cells or the altered state of their environment is capable of persisting for many seconds at a level which causes the rhythmic discharge of impulses from the cells.

10066 P

Relation of Potassium to Family Periodic Paralysis.

GEORGE D. GAMMON.* (Introduced by D. W. Bronk.)

From the Institute of Neurology† and the Medical Clinic of the Hospital, The University of Pennsylvania School of Medicine.

The older literature on family periodic paralysis contains observations which might suggest a relationship of disturbed K metabolism to the attacks. K citrate was thought by Mitchell[1] to have a slightly beneficial effect on the seizures, and McCann[2] found descendants of Mitchell's patient still relying on the drug. Herrington[3] has recently stated that K citrate will prevent an oncoming seizure but will not cure an attack once developed. Furthermore, earlier Japanese investigators[4, 5] showed that certain substances (adrenalin, sugar, insulin) which are now known to lower serum K would induce a seizure.

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* Godey and Seeger Fellow.

† Supported by the Kirby and McCarthy Funds.

[1] Mitchell, J. K., Flexner, S., and Edsall, D. L., *Brain*, 1902, **25**, 109.

We were thus led to examine the level of serum K in a patient with this disease and have found a definite lowering (25 to 30%) during severe attacks. Aitken and Allott[6] independently observed a similar fall during severe seizures, but in milder attacks they found that the decrease was less, and at times not even detectable.

The experiments which are here reported have been conducted on a 16-year-old boy over a period of 15 months. Our purpose has been to gain information concerning the mechanism of the changes in serum K and their relation to the periodic attacks of the disease.

We have found no excessive elimination of K by the kidney preceding the seizures and have, therefore, sought some other cause of the lowering of serum K during the attack. The seizures generally developed at 3 to 4 A. M., and urinary excretion studies showed that at this time the absorption of K from the gut was quite low. Furthermore, the K taken in with the day's diet had been largely eliminated before midnight. We have, therefore, concluded that such an individual has an unusual need for K salts to maintain contractility of certain of his muscles.

Making up this need for K by administration of K salts early in the morning (4 g KCl at 2:30 nightly) has prevented the development of seizures or made them very much less frequent and severe. No such effect, however, has resulted from the administration of similar amounts of K during the day because it was eliminated before being required.

An attack which has already developed can be cured by the administration of K (5 g of KCl orally), an observation which is also reported by Aitken and Allott. In our case this procedure was effective even when the serum level was not low. On the other hand, several other bases involved in muscle metabolism, such as NaCl, $NaHCO_3$, creatine hydrate (15 g orally), and also prostigmin (1.5 mg hypodermically) failed to diminish the weakness.

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[2] McCann, W. S., personal communication.

[3] Herrington, M. S., *J. A. M. A.*, 1937, **108**, 1339.

[4] Shinosaki, T., *Z. Ges. Neurol. u. Psychiatr.*, 1926, **100**, 564.

[5] Yoshimura, K., *Z. f. d. Ges. Exp. Med.*, 1930, **70**, 251.

[6] Aitken, R. S., Allott, E. N., Castleden, L. I. M., and Walker, M., *Clinical Sci.*, 1937, **3**, 47.

The manifestations of this disease are almost surely peripheral in origin for certain muscles are electrically inexcitable during an attack. Attention is thus directed to an abnormality of the muscle itself as a cause of the weakness. The facts so far known could be explained on the assumption that from time to time some alteration in the muscle arises which leads to inexcitability and which then requires additional K for its correction. This need is partially met by diffusion of K from serum into muscle as might be concluded from the fact that serum K falls in a severe attack. The increased requirement is not adequately met, however, unless K can be absorbed from the gut. McEachern's observation[7] that the intravenous administration of KCl in small amounts can partially relieve the seizure without raising the lowered serum K could be explained on the basis of direct diffusion of the administered K into the muscle with resulting relief of the weakness.

In this patient the need for extra K is more or less constant for he develops weakness whenever there is no source of available K in the gut. Indeed the urgency of this need is shown by the observation that the washing out of large amounts of K during a simple water diuresis results in a severe attack. Apparently he is unable to compensate for the reduced concentration of muscle salts which follows ingestion of large quantities of water. In other individuals who have infrequent attacks the metabolic change in muscle postulated above must occur only periodically.

The K taken into the muscle during the attack should, on this hypothesis, be eliminated after the abnormality has been corrected. Experiments to check this point are in progress.

10067 P

Immunological Properties of a Sonic Extract of Pneumococci.

LESLIE A. CHAMBERS AND A. J. WEIL. (Introduced by D. W. Bronk.)

From the Eldridge Reeves Johnson Foundation, University of Pennsylvania, Philadelphia, and the Research Department, Lederle Laboratories, Inc., Pearl River, New York.

Formalin-killed, young cultures of pneumococci have been found by one of us (Weil)[1] to evoke infiltration when injected intradermally into rabbits, the infiltration being type-specifically neutralizable by antipneumococcal sera. Subsequent experience has shown that for a given amount of antigen the extent of the neutralizing effect is proportional to the antibody content of the antisera. In the absence of information as to the nature of the active principle it was tentatively called "the irritating substance". Type specific carbohydrates alone do not produce infiltration. On the other hand, since there is type-specificity in neutralization with antisera, it must be concluded that the specific soluble substance determines the specificity of the reaction. Therefore, it seemed possible that the "irritating substance" might be related to the long sought complete antigen of the pneumococci.

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[7] McEachern, D., personal communication.

[1] Weil, A. J., and Phillips, S. W., *J. Immunol.*, 1937, **33**, 149.

Because of the apparent lability of the "irritating substance" toward most manipulative procedures, attempts to obtain further knowledge of its nature by chemical methods were unsuccessful. Recently, however, we have obtained the active principle by sonic disintegration of the pneumococci.

The magnetostriction oscillator used in this work was similar to that previously employed by Chambers and Flosdorf[2] in the sonic extraction of labile antigenic substances from *Strep. hemolyticus* and *Eberthella typhosa*. Suspensions of the bacteria were exposed in lots of about 40 ml to 9000 c.p.s. vibrations of sufficient intensity to cavitate the liquid vigorously. Temperature of the cultures was so controlled by water cooling that it did not rise above 20°C at any time.

Experiments were done with pneumococci of types 1 and 2, grown for 6 hours at 37°, and with formalinized vaccines from 6-hour cultures of pneumococci of Types 1, 2, 3, 7, and 14. All strains used were highly virulent and the cultures and vaccines were strictly Gram-positive. Heavy suspensions containing 20 to 100 billion organisms per cc were used. All types gave essentially the same results and can therefore be reported together. With the oscillator we used, 60 to 75 minutes of vibration were found necessary to destroy all cocci. Progress of the dissolution could be followed on smears made at different times during the treatment. The cocci started to turn Gram-negative after 15 minutes. More and more amorphous detritus appeared until finally almost no cocci remained. Simultaneously the suspensions gradually cleared, assuming the translucence of a typical protein solution. During strong centrifugation the detritus settled down as a whitish sediment, the supernatant liquid being transparent but showing a pronounced Tyndall effect.

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[2] Chambers, L. A., and Flosdorf, E. W., PROC. SOC. EXP. BIOL. AND MED., 1936, **34**, 631.

This supernatant material gave strong precipitation up to 1/20,000 dilution with homologous rabbit and horse antipneumococcal sera. With heterologous antipneumococcal sera there was often some precipitation but only up to dilutions of 1/200 to 1/400. It is not yet possible to make any statement as to the nature of the species specific reaction, but there is no doubt that the material reacts predominantly type-specifically. There was no great loss of activity upon passage of the supernatant liquid through Berkefeld "N" filters, and the resultant colloidal solutions, completely free from formed cellular elements, gave typical type-specifically neutralizable skin reactions in rabbits. It is difficult to estimate the quantities of irritating substances present in the solutions but the reactivity was definitely less in the treated material as compared with the original suspension of cells.

The amorphous sediment remaining in the centrifuge tubes gave no skin reaction whatever.

When the active solutions were frozen their activity remained unimpaired during several weeks of preservation. Some material flocculated out during storage, but it could be removed by centrifuging and was not skin reactive, while the final supernatant solution retained all its reactive qualities.

We tried to evoke antibody formation in rabbits with material of this kind obtained from living pneumococci of Types 1 and 2, and from formalinized vaccines of Types 2, 3, and 7. Altogether 20 rabbits were injected intravenously with increasing doses for 5 weeks. No antibody formation was observed *in vitro*.

We have, therefore, a material which shows one quality more than the S substance, namely skin reactivity, but which does not evoke antibody formation in the rabbit. The faculty of giving skin reactions in the sense defined above is evidently not confined to the complete type-specific antigen of the pneumococcus, but is present at a level of organization intermediate between it and the carbohydrate. These facts are summarized in Table I.

TABLE I.

Stages of Type-specific Pneumococcal Reactivity.

	Reactivity in test tube	Reactivity in skin of rabbits	Antibody formation in rabbits
Specific soluble substance	+	—	—
"Irritating substance"	+	+	—
Whole pneumococcus	+	+	+

The data presented show that intense sonic vibration disintegrates pneumococci into a colloidal solution and an amorphous remainder, and that the solution contains a product which reacts predominantly type-specifically *in vitro* and gives type-specifically neutralizable reaction in the skin of rabbits. It does not elicit antibody formation in these animals.

We are continuing this investigation especially to obtain the active principle in a more purified state. It will be interesting to determine what relation it bears to the "conjugated carbohydrate" recently described by Sevag.[3]

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[3] Sevag, M. G., *Science*, 1938, **87**, 304.

SECRETARY'S REPORT

April 1, 1937 to April 1, 1938

Dr. H. B. Lewis has given ten years of loyal, devoted service as editor of the PROCEEDINGS. He believes in the principle of rotation in the Board of Editors, and asks to be relieved of his duties. The Council wishes to express its high appreciation of his extraordinarily fine service, and to thank him for helping so markedly in the development of the PROCEEDINGS.

In addition to the data submitted below for the information of members, as well as for record, the Secretary wishes to stress the following:

Manuscripts.

1. The chief cause of delay in publication is the lack of designation whether a manuscript is preliminary or complete.
2. Only confirmatory manuscripts which deal with distinctly controversial subjects are acceptable.
3. A sponsor is expected to assume responsibility not only for the standards and presentation of work, but also for the payment of costs.
4. An author is expected to exercise the greatest care in precision of expression of his ideas, to avoid gross inaccuracies, vulgarisms, looseness of expression and verboseness.
5. Abbreviations should conform to U. S. Bureau of Standards or to the J. B. C.
6. Forty-five per cent of manuscripts published were preliminary, 55% complete. Ten per cent of manuscripts received were rejected, 20% returned for radical revision.

Membership. Unanimous approval of the National Membership Committee constitutes election. If the vote is not unanimous but more than half, then the application shall be referred to the Council at its annual meeting.

Proceedings Wanted. The thanks of the Society are due to the following members for their prompt sending of back numbers required to complete sets of the Proceedings for libraries: Doctors A. F. Coca, W. H. Cole, D. L. Fox, E. L. Gustus, A. T. Henrici, L. Leiter, F. H. Pratt, C. Reynolds, C. L. A. Schmidt, G. V. Smith, H. Tauber, S. A. Thayer, C. V. Weller, and the Library of the New York Academy of Medicine.

We are in special need of earlier numbers. Members are requested to spare the folowing:

Year	*Month*	*Volume*	*No.*
1915	May	12	8
	October	13	1
	November	13	2
	December	13	3
1916	January	13	4
	February	13	5
	March	13	6
1921	November	19	2

	December	19	3
1922	April	19	7
	May	19	8
	December	20	3
1930	October	28	1

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1919-20	G. N. Calkins	G. B. Wallace	" "	
1920-21	G. N. Calkins	G. B. Wallace	" "	
1921-22	G. B. Wallace	J. W. Jobling	" "	
1922-23	G. B. Wallace	J. W. Jobling	" "	
1923-24	H. C. Jackson	J. W. Jobling	V. C. Myers	
1924-25	H. C. Jackson	J. W. Jobling	A. J. Goldforb	
1925-26	J. W. Jobling	S. R. Benedict	" "	

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SECTIONAL MEETINGS AND MEMBERSHIP

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Chairman: E. Muntwyler. Secretary: H. D. Green. Members: 42.
Meetings: Western Reserve University, October 15, 1937.
November 12, 1937
January 14, 1938
May 13, 1938

District of Columbia

Chairman: M. E. Maver. Secretary: C. E. Leese. Members: 29.
Meetings: National Institute of Health, November 23, 1937
February 1, 1938

Illinois

Chairman: C. A. Dragstedt. Secretary: L. A. Crandall, Jr. Members: 125.
Meetings: Northwestern University, December 23, 1937
University of Illinois Medical School, March 15, 1938
University of Chicago, May 10, 1938

Iowa

Chairman: H. P. Smith. Secretary: W. R. Ingram. Members: 36.
Meetings: State University of Iowa, November 2, 1937
February 8, 1938
May 17, 1938

Minnesota

Chairman: F. H. Scott. Secretary: F. H. Scott. Members: 46.
Meetings: University of Minnesota, November 17, 1937
December 15, 1937
March 2, 1938
April 20, 1938

Missouri

Chairman: G. H. Bishop. Secretary: A. B. Hertzman. Members: 54.
Meetings: St. Louis University Medical School, October 13, 1937
Washington University Medical School, November 10, 1937
St. Louis University Medical School, December 8, 1937
Washington University Medical School, March 9, 1938
St. Louis University Medical School, April 13, 1938
Washington University Medical School, May 11, 1938

New York

Chairman: H. S. Gasser. Secretary: I. Greenwald. Members: 792.
Meetings: New York Academy of Medicine, October 20, 1937
November 17, 1937
December 15, 1937
January 19, 1938
February 16, 1938
March 16, 1938
April 20, 1938
May 18, 1938

Pacific Coast

Chairman: A. W. Meyer. Secretary: C. Weiss. Members: 93.
Meetings: Stanford University, October 16, 1937
University of California Medical School, December 8, 1937
Stanford University Medical School, February 2, 1938
Mount Zion Hospital, March 9, 1938
University of California, May 7, 1938

Peiping (China)

Chairman: S. H. Liu. Secretary: C. H. Hu. Members: 35.
Meetings: Peiping Union Medical College, October 13, 1937
December 22, 1937
April 6, 1938

Southern

Chairman: H. S. Mayerson. Secretary: R. Ashman. Members: 37.
Meetings: Municipal Auditorium, New Orleans, November 30, 1937
Tulane University, February 25, 1938
May 13, 1938

Southern California

Chairman: G. H. Ball. Secretary: C. H. Thienes. Members: 36.
Meetings: University of Southern California, October 20, 1937
Scripps Institution, December 11, 1937
Los Angeles Co. General Hospital, February 12, 1938
University of California at Los Angeles, May 2, 1938

Western New York

Chairman: W. J. Atwell. Secretary: R. R. Humphrey. Members: 57.
Meetings: Clifton Springs Sanitarium, October 30, 1937
University of Buffalo, December 11, 1937
University of Rochester Medical School, February 12, 1938
Syracuse University, May 21, 1938

Wisconsin

Chairman: W. E. Sullivan. Secretary: O. O. Meyer. Members: 37.
Meetings: University of Wisconsin, May 10, 1938

Membership

Members, March 31, 1937	1346	
Elected during year	83	
Honorary Members	8	
Total		1437
Resignations	5	
Deaths	8	
Arrears	5	
		18
Total Membership, March 31, 1938		1419

	1928	1938
Membership	936	1419

Subscriptions, March 31, 1938 586

Deaths of Members

The Council records with regret the deaths of the following members: Doctors H. H. Collins, H. H. Donaldson, L. Gross, J. A. Hawkins, E. P. Lyon, A. R. Mandel, J. L. Miller and H. B. Myers.

A. J. Goldforb,
Secretary.

TREASURER'S REPORT
April 1, 1937 to April 1, 1938

The Treasurer desires to inform members that in spite of increased cost of printing, it is possible to *reduce* the cost to authors for the coming year, viz.,

Excess space and tables will be reduced from 40 to 20% of our cost.

Cuts will be reduced from 75 to 50% of our cost.

Members are allowed two printed pages a year without cost. At the low price of the Proceedings to members (two volumes a year for four dollars) it is not financially possible to transfer the cost of excess space from one member to another, or to a non-member.

Receipts

Balance on Hand, April 1, 1937		$ 6,337.79
Income, 1937-38		
Dues	$ 5,785.06	
Reprints	2,902.39	
Excess Space	1,257.56	
Cuts	826.80	
Changes	39.20	
Subscriptions	3,858.66	
Back Numbers Sold	313.85	
Interest (special accounts)	64.26	
Total Annual Income		$15,047.78
Endowment Fund temporarily in Corn Exchange Bank		1,811.67
Total Cash Available, April 1, 1937-April 1, 1938		$23,197.24

Disbursements

Publication Cost of Proceedings:		
Printing	$ 7,424.65	
Reprints	2,829.63	
Cuts	731.73	
		$10,986.01
Administrative Expenses:		
Office Supplies, Postage and Telephone	$ 694.37	
Salary	2,100.00	
Storage and Insurance	75.67	
Miscellaneous	207.77	
		3,077.81
Total Annual Disbursements		$14,063.82
Cash Balance, April 1, 1938		9,133.42
Total		$23,197.24

Summary

Income (net)	$15,047.78
Disbursements (net)	14,063.82
Surplus	$ 983.96

Accounts payable—none
Accounts receivable—$675.18

Funds

Endowment Fund

April 1, 1937	$16,005.89	
Interest to April 1, 1938	624.93	
		$16,630.82
Invested in N. Y. Title and Mortgage Co.	$ 5,940.00	
Title Guarantee and Trust Co.	2,000.00	
Lawyers Mortgage Co.	1,500.00	
R. R. Federal Savings and Loan	25.25	
Bowery Savings Bank	1,632.41	
Industrial Bonds	3,721.49	
Temporarily deposited in Corn Exchange Bank	1,811.67	
		$16,630.82

Surplus Fund

April 1, 1937	$10,064.44	
Interest to April 1, 1938	439.83	
		$10,504.27
Invested in Title Guarantee and Trust Co.	$ 2,900.00	
R. R. Federal Savings and Loan	959.33	
Bowery Savings Bank	770.75	
Industrial Bonds	5,874.19	
		$10,504.27

Life Membership Fund

Invested in R. R. Federal Savings and Loan	$ 75.00

Auditors' Report

May 11, 1938.

We have examined the Treasurer's report and have verified the statements therein against the books of the Society. We are satisfied that the records of financial transactions are in good order and accurately kept.

In view of the legal incorporation of the Society it is recommended that beginning with next year, and every third year thereafter, the auditing committee be authorized to employ a certified public accountant to certify the Treasurer's records. The charge for this service, which is not to exceed $125, is to be carried by the Society. This recommendation is for the protection of the Secretary-Treasurer and the auditors of the Society.

(Signed) Homer W. Smith
Philip E. Smith

MEMBERS' LIST

HONORARY MEMBERS

Flexner, Simon	Rockefeller Institute
Howell, William H.	Johns Hokins Univ.
Lee, F. S.	New York City
Lombard, Warren P.	Univ. of Michigan
Porter, William	Harvard Univ.
Richet, Charles	Paris, France
Von Muller, Friedrich	Munich, Germany
Wilson, Edmund B.	Columbia Univ.

MEMBERS

Abramson, H. A.	Coll. Physicians and Surgeons, New York
Abt, Arthur F.	Northwestern Univ.
Adams, A. Elizabeth	Mount Holyoke Coll.
Adams, William E.	Univ. of Chicago
Addis, Thomas	Med., Stanford Univ.
Adolph, E. F.	Univ. of Rochester Med.
Adolph, W. H.	Peiping, China
Alexander, Harry L.	Washington Univ.
Allen, Bennet M.	Univ. of California, Los Angeles
Allen, Edgar	Yale Univ.
Allen, Ezra	N. Y. Homeopathic Med.
Allen, William F.	Univ. of Oregon
Alles, G. A.	Los Angeles, California
Alsberg, Carl L.	Univ. of California
Alt, Howard L.	Northwestern Univ. Med.
Althausen, T. L.	Univ. of California Med.
Altschule, M. D.	Beth Israel Hospital, Boston
Alvarez, Walter C.	Mayo Clinic
Alving, A. S.	Univ. of Chicago
Amberg, Samuel	Mayo Clinic
Amberson, W. R.	Univ. of Maryland Med.
Amoss, Harold L.	Rockefeller Institute
Anderson, Dorothy H.	Coll. of Physicians and Surgeons
Anderson, H. H.	Chicago, Ill.
Anderson, John E.	Univ. of Minnesota
Anderson, John F.	E. R. Squibb & Son
Anderson, Rudolph J.	Yale Univ.
Anderson, William E.	Springfield, Mass.
Andrews, Edmund	Univ. of Chicago
Anson, Mortimer L.	Rockefeller Institute, Princeton, N. J.
Antopol, William	Beth Israel Hospital, Newark, N. J.
Armstrong, Charles	National Inst. of Health, Washington
Arnold, Lloyd	Univ. of Illinois

Aronson, J. D.	Henry Phipps Institute
Asdell, S. A.	Cornell Univ.
Asher, Leon	Berne, Switzerland
Ashman, Richard	Louisiana State Univ. Medical Center
Asmundson, V. S.	Univ. of California
Atchley, D. W.	Presbyterian Hospital, New York City
Atkinson, H. V.	Vermillion, S. D.
Atwell, Wayne J.	Univ. of Buffalo, N. Y.
Aub, Joseph C.	Huntington Memorial Hospital, Boston
Auer, John	St. Louis Univ.
Austin, J. Harold	Univ. of Pennsylvania
Avery, O. T.	Rockefeller Institute, N. Y. City
Avery, Roy C.	Vanderbilt Univ.
Aycock, W. L.	Harvard Med.
Babkin, Boris P.	McGill Univ.
Bachem, Albert	Univ. of Illinois Medical Coll.
Baehr, George	Mt. Sinai Hospital, N. Y. City
Bagg, Halsey J.	Memorial Hospital, N. Y. City
Bahrs, Alice M.	Portland, Ore.
Bailey, Cameron V.	N. Y. Post-Graduate Med.
Baitsell, George A.	Yale Univ.
Baker, Lillian E.	Rockefeller Institute
Bakwin, Harry	N. Y. Univ. and Bellevue Medical Coll.
Baldwin, Francis M.	Univ. of S. Calif.
Ball, G. H.	Univ. of California, Los Angeles
Ball, H. A.	San Diego, Calif.
Balls, A. K.	U. S. Dept. of Agriculture
Banta, A. M.	Carnegie Institution, Cold Spring Harbor
Banting, Frederick G.	Univ. of Toronto
Barach, Alvin L.	Coll. of Physicians and Surgeons
Barber, W. Howard	New York Univ. Med.
Barbour, Henry G.	Yale Univ.
Barer, Adelaide P.	State Univ. of Iowa
Barlow, O. W.	Rensselaer, N. Y.
Barnett, George D.	Stanford Univ.
Barr, David P.	Washington Univ.
Barron, E. S. G.	Univ. of Chicago
Barth, L. G.	Columbia Univ.
Bartley, S. H.	Washington Univ.
Bass, Charles	Tulane Univ.
Bast, T. H.	Univ. of Wisconsin
Bates, R. W.	Carnegie Institution of Washington
Bauer, J. H.	Rockefeller Institute
Bauman, Louis	Presbyterian Hospital, N. Y. City
Baumann, E. J.	Montefiore Hospital, N. Y. City
Baumberger, J. Percy	Stanford Univ.
Bayne-Jones, S.	Yale Univ.
Bazett, H. C.	Univ. of Pennsylvania
Bean, John W.	Univ. of Michigan
Beard, H. H.	Louisiana State Univ. Medical Center

Beard, J. W.	Rockefeller Institute
Beard, P. J.	Stanford Univ.
Beck, Claude S.	Western Reserve Univ.
Becker, E. R.	Iowa State Coll.
Beckman, Harry	Marquette Univ. Med.
Beckwith, T. D.	Univ. of California, Los Angeles
Behre, Jeannette A.	Cornell Univ. Medical Coll.
Belding, David L.	Boston Univ.
Bellamy, Arthur W.	Univ. of California, Los Angeles
Berg, B. N.	Columbia Univ.
Berg, C. P.	State Univ. of Iowa
Berg, William N.	New York City
Bergeim, Olaf	Univ. of Illinois
Bergey, David H.	National Drug Co., Philadelphia
Bergmann, Max	Rockefeller Institute
Bernhard, Adolph	Lenox Hill Hospital, N. Y. City
Bernthal, T. G.	Univ. of Michigan
Berry, George P.	Univ. of Rochester Med.
Beutner, R.	Hahnemann Medical College, Philadelphia
Bieter, Raymond N.	Univ. of Minnesota
Bills, C. E.	Mead, Johnson and Co., Evansville, Ind.
Bing, Franklin C.	American Med. Assn., Chicago
Binger, Carl A. L.	Rockefeller Institute, N. Y. City
Birkhaug, Konrad E.	Geophysic Institute, Bergen, Norway
Bishop, George H.	Webster Groves, Mo.
Bishop, Katharine S.	Univ. of California
Blackfan, K. D.	Harvard Med. School
Blair, John E.	Hospital for Joint Diseases, N. Y. City
Blake, F. G.	Yale Univ.
Blakeslee, Albert F.	Station for Exp. Evolution, Cold Spring Harbor, N. Y.
Blalock, Alfred	Vanderbilt Univ. Med.
Blatherwick, Norman R.	Metropolitan Life Insurance Co., N. Y. City
Blau, Nathan F.	Cornell Univ. Medical Coll.
Blinks, L. R.	Stanford Univ.
Bliss, Sidney	Tulane Univ.
Bloch, Robert G.	Univ. of Chicago
Block, Richard J.	N. Y. State Psychiatric Institute
Bloom, William	Univ. of Chicago
Bloomfield, A. L.	Stanford Univ. Med.
Bloor, W. R.	Univ. of Rochester
Blount, R. F.	Univ. of Minnesota
Blum, Harold F.	Univ. of California
Blumgart, H. L.	Beth Israel Hospital, Boston
Bock, Joseph C.	Marquette Univ.
Bodansky, A.	Hospital for Joint Diseases, N. Y. City
Bodansky, Meyer	John Sealy Hospital, Galveston, Texas
Bodansky, Oscar	New York Univ.
Bodine, J. H.	State Univ. of Iowa
Bodo, Richard	N. Y. Univ. and Bellevue Medical Coll.
Bogen, Emil	Olive View, Calif.
Boissevain, Charles H.	Colorado College

Bollman, Jesse L.	Mayo Clinic
Boor, Alden K.	Univ. of Chicago
Boothby, Walter M.	Kahler Hospital, Rochester, Minn.
Boots, Ralph H.	Presbyterian Hospital, N. Y. City
Borsook, Henry	California Institute of Technology
Bowen, B. D.	Buffalo General Hospital
Boyd, Eldon M.	Queens University, Canada
Boyd, Theo. E.	Loyola Univ.
Boyden, E. A.	Univ. of Minnesota Med.
Bradford, William L.	Univ. of Rochester
Bradley, H. C.	Univ. of Wisconsin
Brand, Erwin	N. Y. State Psychiatric Institute
Branham, Sara E.	National Inst. of Health, Washington
Brewer, Robert K.	Syracuse Univ.
Briggs, A. P.	Univ. of Georgia
Brodie, Maurice	Providence Hospital, Detroit, Mich.
Bronfenbrenner, J.	Washington Univ.
Bronk, D. W.	Univ. of Pennsylvania
Brooks, Clyde	Louisiana State Univ. Medical Center
Brooks, Matilda M.	Univ. of California
Brooks, S. C.	Univ. of California
Broun, G. O.	St. Louis Univ. Med.
Brown, J. Howard	Johns Hopkins Univ.
Brown, John B.	Ohio State Univ.
Brown, L. A.	Transylvania Coll., Lexington, Ky.
Brown, Rachel	N. Y. State Dept. of Health
Brown, Wade H.	Rockefeller Institute, Princeton
Browne, J. S. L.	Royal Victoria Hosp., Montreal
Bruger, Maurice	N. Y. Post-Graduate Med.
Brunschwig, Alexander	Univ. of Chicago
Buchanan, Robert E.	Iowa State Coll.
Buchbinder, W. C.	Michael Reese Hospital, Chicago
Buell, Mary V.	Johns Hopkins Univ.
Bulger, H. A.	Washington Univ.
Bullowa, J. G. M.	Harlem Hospital, N. Y. City
Bunting, C. H.	Univ. of Wisconsin
Bunting, R. W.	Univ. of Michigan
Burch, John C.	Vanderbilt Univ. Med.
Burdon, Kenneth L.	Louisiana State Univ.
Burget, G. E.	Univ. of Oregon Med.
Burky, Earl L.	Johns Hopkins Hospital
Burns, Robert K., Jr.	Univ. of Rochester
Burr, George O.	Univ. of Minnesota
Burr, Harold S.	Yale Univ.
Burrows, M. T.	Pasadena, Calif.
Butcher, E. O.	Hamilton Coll.
Butt, E. M.	Univ. of Southern California
Butts, Joseph S.	Univ. of Southern California
Byerly, T. C.	U. S. Animal Exp. Farm, Beltsville, Md.
Byrne, Joseph	Fordham Univ

Calkins, Gary N.	Columbia Univ.
Cameron, A. T.	Univ. of Manitoba
Cannan, Robert K.	N. Y. Univ. and Bellevue Medical Coll.
Cannon, Paul R.	Univ. of Chicago
Cannon, Walter B.	Harvard Med. School
Cantarow, Abraham	Jefferson Medical Coll.
Carey, E. J.	Marquette Univ.
Carlson, A. J.	Univ. of Chicago
Carmichael, E. B.	Univ. of Alabama Med.
Carmichael, L.	Univ. of Rochester
Casey, Albert E.	St. Louis Univ.
Cash, James R.	Univ. of Virginia
Casida, L. E.	Univ. of Wisconsin
Cattell, McKeen	Cornell Univ. Medical Coll.
Caulfeild, A. H. W.	Univ. of Toronto
Cecil, R. L.	Cornell Univ. Medical Coll.
Cerecedo, L. R.	Fordham Univ.
Chace, Arthur F.	N. Y. Post-Graduate Medical Coll.
Chaikoff, I. L.	Univ. of California
Chambers, Robert	New York Univ.
Chambers, William H.	Cornell Univ. Medical Coll.
Chang, Hsi Chun	Peiping Union Medical Coll.
Chang, Hsiao-Chien	Hunan, China
Chargaff, Erwin	Coll. of Physicians and Surgeons
Charipper, H. A.	New York Univ.
Cheer, S. N.	Central Hospital, Nanking, China
Chen, Graham M.	Peiping Union Medical Coll.
Chen, K. K.	Eli Lilly and Co., Indianapolis
Chen, T. T.	Peiping Union Medical Coll.
Cheney, R. H.	Long Island Univ.
Chidester, F. E.	Newark Valley, N. Y.
Child, C. M.	Stanford Univ.
Chittenden, R. H.	Yale Univ.
Chouke, K. S.	Univ. of Colorado
Chow, B. F.	Peiping Union Med. Coll.
Christensen, K.	St. Louis Univ.
Christian, Henry A.	Peter Bent Brigham Hospital
Christman, Adam A.	Univ. of Michigan
Chu, F. T.	Peiping Union Medical Coll.
Clark, A. J.	University of Edinburgh, Scotland
Clark, Ada R.	Coll. of Physicians and Surgeons
Clark, Guy W.	Lederle Lab., Pearl River, N. Y.
Clark, P. F.	Univ. of Wisconsin
Clarke, Hans T.	Coll. of Physicians and Surgeons
Claude, A.	Rockefeller Inst.
Clausen, H. J.	Univ. of Colorado Med.
Claussen, S. W.	Strong Memorial Hospital, Rochester, N. Y.
Clawson, Benjamin J.	Univ. of Minnesota
Clifton, Charles E.	Stanford Univ.
Clowes, G. H. A.	Eli Lilly and Co., Indianapolis, Indiana

Coca, A. F.	Oradell, N. J.
Coggeshall, L. T.	Rockefeller Foundation
Coghill, G. E.	Gainesville, Fla.
Cohen, Barnett	Johns Hopkins Med.
Cohen, Martin	N. Y. Post-Graduate Med.
Cohen, Milton B.	St. Alexis Hospital, Cleveland
Cohn, A. E.	Rockefeller Institute, N. Y. City
Cohn, Isidore	New Orleans, La.
Cole, Arthur G.	Univ. of Illinois Med.
Cole, E. C.	Williams Coll.
Cole, Harold H.	Univ. of California, Davis
Cole, L. J.	Univ. of Wisconsin
Cole, Rufus I.	Rockefeller Institute, N. Y. City
Cole, Warren H.	Univ. of Illinois Med.
Cole, William H.	Rutgers Univ.
Coleman, Warren	New York Univ. Medical Coll.
Collens, William S.	Brooklyn, N. Y.
Collett, Mary E.	Western Reserve Univ.
Collins, D. A.	Temple Univ.
Collier, William D.	St. Elizabeth's Hospital, Youngstown, O.
Collip, J. B.	McGill Univ.
Compere, E. L.	Univ. of Chicago
Conklin, E. G.	Princeton Univ.
Connor, Charles L.	Univ. of California Med.
Cook, Donald H.	School of Tropical Medicine, San Juan, P. R.
Cooke, J. V.	Washington Univ.
Coombs, Helen C.	N. Y. Homeopathic Medical Coll.
Cope, O. M.	N. Y. Homeopathic Med.
Copenhaver, W. M.	Columbia Univ.
Corey, E. L.	Univ. of Va.
Cori, Carl F.	Washington Univ.
Corley, Ralph C.	Purdue Univ.
Corner, George W.	Univ. of Rochester
Corper, H. J.	National Jewish Hospital, Denver, Colo.
Co Tui	N. Y. Univ. Medical Coll.
Coulter, Calvin B.	Columbia Univ.
Cowdry, E. V.	Washington Univ.
Cowgill, George R.	Yale Univ.
Cowie, D. M.	Univ. of Michigan
Cox, Herald R.	U. S. Public Health Inst., Hamilton, Mont.
Cox, Warren M., Jr.	Mead Johnson Co.
Craig, C. F.	Tulane Univ.
Cram, Eloise B.	Nat. Inst. of Health, Washington
Crampton, C. Ward	N. Y. Post-Graduate Med.
Crandall, L. A., Jr.	Northwestern Univ.
Crile, George W.	Western Reserve Univ.
Crittenden, Phoebe J.	George Washington Univ.
Crohn, Burrill B.	Mt. Sinai Hospital, N. Y. City
Csonka, F. A.	U. S. Dept. of Agriculture, Washington, D. C.
Cullen, Glenn E.	Children's Hospital, Cincinnati, O.

Cummins, Harold	Tulane Univ.
Cunningham, Bert	Duke Univ.
Cunningham, R. S.	Albany Med. Coll.
Curtis, G. M.	Ohio State Univ.
Curtis, Maynie R.	Columbia Univ.
Cushing, Harvey W.	Yale Univ.
Cutler, Elliott C.	Peter Bent Brigham Hospital, Boston

Dack, Gail M.	Univ. of Chicago
Dakin, H. D.	Ossining, N. Y.
D'Amour, F. E.	Univ. of Denver
Danforth, Charles H.	Stanford Univ.
Daniel, J. Frank	Univ. of California
Daniels, Amy L.	Univ. of Iowa
Danzer, Charles S.	N. Y. Homeopathic Medical Coll.
Dautrebande, Lucien	Univ. of Liege, Belgium
Davenport, C. B.	Sta. for Exp. Evolution, Cold Spring Harbor, N. Y.
Davenport, H. A.	Northwestern Univ. Med.
Davis, D. J.	Wilmette, Illinois
Davis, M. E.	Univ. of Chicago
Dawson, James A.	Coll. of the City of N. Y.
Dawson, M. H.	Columbia Univ.
Dawson, W. T.	Univ. of Texas Med.
Day, A. A.	Northwestern Univ. Med.
Day, Paul L.	Univ. of Arkansas Med.
Decherd, George M.	Louisiana State Univ.
DeEds, Floyd	Stanford Univ. Med.
DeGraff, A. C.	N. Y. Univ. Med.
Dennis, E. W.	Amer. Univ. of Beirut
DeRenyi, G. S.	Univ. of Pennsylvania
DeSavitsch, Eugene	New York City
Detwiler, S. R.	Columbia Univ.
Deuel, Harry J., Jr.	Univ. of S. Calif. Med.
Dick, George F.	Univ. of Chicago
Dickson, E. C.	Stanford Univ. Med.
Dieckmann, W. J.	Univ. of Chicago
Dienes, Louis	Massachusetts General Hospital, Boston
Dieuaide, Francis R.	Peiping Union Medical Coll.
Doan, Charles A.	Ohio State Univ.
Dochez, A. R.	Presbyterian Hospital, N. Y. City
Dock, William	Stanford Univ.
Doisy, Edward A.	St. Louis Univ.
Dolley, W. L., Jr.	Univ. of Buffalo
Dominguez, R.	St. Luke's Hospital, Cleveland
Domm, L. V.	Univ. of Chicago
Donaldson, J. C.	Univ. of Pittsburgh
Dooley, M. S.	Syracuse Univ.
Doull, J. A.	Western Reserve Univ.
Downes, Helen R.	Memorial Hospital, N. Y. City
Drabkin, D. L.	Univ. of Pennsylvania

Dragstedt, Carl A.	Northwestern Univ.
Dragstedt, Lester R.	Univ. of Chicago
Draper, George W.	Columbia Univ.
Draper, William B.	Univ. of Colorado
Dresbach, M.	Albany, N. Y.
Drury, D. R.	Univ. of S. Calif. Med.
Dubin, Harry E.	N. Y. City
DuBois, E. F.	Cornell Univ. Medical Coll.
DuBois, F. S.	Hartford, Conn.
Duggar, B. M.	Univ. of Wisconsin
Dukes, H. H.	Cornell Univ.
Dunn, Leslie C.	Columbia Univ.
Dunn, Max	Univ. of California, Los Angeles
Duran-Reynals, F.	Rockefeller Institute
Dusser de Barenne, J. G.	Yale Univ.
Dutcher, R. Adams	Pennsylvania State Coll.
Duval, C. W.	Tulane Univ.
Du Vigneaud, Vincent	George Washington Univ. Med.
Dye, Joseph A.	Cornell Univ. Med.
Dyer, Helen M.	George Washington Univ. Med.

Earle, Wilton R.	National Inst. of Health, Washington
Eastman, N. J.	Johns Hopkins Univ.
Eberson, Frederick	San Francisco, Calif.
Ecker, E. E.	Western Reserve Univ.
Eckstein, Henry C.	Univ. of Michigan
Eddy, Walter H.	Columbia Univ.
Edmunds, C. W.	Univ. of Michigan
Edwards, D. J.	Cornell Univ. Medical Coll.
Edwards, John G.	Univ. of Buffalo
Eggston, Andrew A.	N. Y. Manhattan Eye, Ear Hospital
Ellis, Max M.	Univ. of Missouri
Elsberg, Charles A.	Neurological Institute, N. Y. City
Elser, W. J.	Cornell Univ. Medical Coll.
Emerson, G. A.	West Virginia Univ. Med.
Emery, F. E.	Univ. of Buffalo
Emge, L. A.	Stanford Univ.
Enders, J. F.	Harvard Univ.
Engle, E. T.	Columbia Univ.
Epstein, A. A.	Mt. Sinai Hospital, N. Y. City
Erlanger, Joseph	Washington Univ.
Ernstene, Arthur C.	Cleveland Clinic
Essex, Hiram E.	Mayo Clinic
Evans, Alice C.	National Inst. of Health, Washington
Evans, Gerald T.	Yale Univ.
Evans, Herbert M.	Univ. of California
Everett, M. R.	Univ. of Oklahoma Med.
Ewing, James	Cornell Univ. Medical Coll.
Eyster, J. A. E.	Univ. of Wisconsin

Faber, Harold K.	Stanford Univ. Med.
Fahr, George	Univ. of Minnesota
Falk, I. S.	Washington, D. C.
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Farmer, Chester	Northwestern Univ.
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Fearing, Franklin	Univ. of California, Los Angeles
Feil, Harold	Western Reserve Univ.
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Fenn, Wallace O.	Univ. of Rochester Med.
Ferguson, John H.	Univ. of Michigan
Ferguson, J. K. W.	Ohio State Univ.
Ferraro, Armando	N. Y. State Psychiatric Institute
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Field, John, II	Stanford Univ.
Figge, F. H. J.	Univ. of Maryland Med.
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Firor, W. M.	Johns Hopkins Univ.
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Fischer, E.	Medical Coll. of Va.
Fischer, Martin H.	Univ. of Cincinnati
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Fitch, C. P.	Univ. of Minnesota
Fitz, Reginald	Peter Bent Brigham Hospital
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Fleisher, Moyer S.	St. Louis Univ.
Flinn, Frederick B.	Columbia Univ.
Florence, Laura	N. Y. Homeopathic Med.
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Forbes, Henry	Milton, Mass.
Forkner, Claude E.	New York City
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Freedlander, S. O.	Western Reserve Univ.
Freedman, Louis	New York City
Freeman, R. G., Jr.	Boston, Mass.
Freudenberger, C. B.	Univ. of Utah
Freund, Jules	Cornell Univ. Medical Coll.
Fridericia, L. S.	Univ. of Copenhagen, Denmark
Friedemann, T. E.	Univ. of Chicago
Friedman, M. H.	Univ. of Pennsylvania
Frobisher, Martin, Jr.	Baltimore, Md.
Fry, Henry J.	Cornell Medical Coll.

Fulton, John F.	Yale Med.
Funk, Casimir	Paris, France
Furth, Jacob	Cornell Univ. Medical Coll.
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Gardner, William U.	Yale Univ.
Garrey, Walter E.	Vanderbilt Univ.
Gaskell, John F.	Cambridge, England
Gasser, Herbert S.	Rockefeller Institute
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Gay, F. P.	Columbia Univ.
Geiling, E. M. K.	Univ. of Chicago
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Gelarie, Arnold J.	New York City
Gellhorn, Ernst	Univ. of Illinois Medical Coll.
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Gerard, R. W.	Univ. of Chicago
Gerstenberger, H. J.	Western Reserve Univ.
Gesell, Robert A.	Univ. of Michigan
Gettler, A. O.	N. Y. Univ. Medical Coll.
Geyelin, H. R.	Columbia Univ.
Gibbs, O. S.	Univ. of Tenn.
Gibson, R. B.	State Univ. of Iowa
Gies, William J.	Columbia Univ.
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Gilman, Alfred	Yale Univ.
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Goldfarb, Walter	Floral Park, L. I.
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Goldring, W.	N. Y. Univ. Med.
Goldschmidt, Richard	Univ. of California
Goldschmidt, Samuel	Univ. of Pennsylvania
Goodman, L.	Yale Univ.

Goodner, Kenneth	Rockefeller Institute
Gortner, R. A.	Univ. of Minnesota
Goss, C. M.	Columbia Univ.
Goss, Harold	Univ. of California
Grace, A. W.	Cornell Med. Coll.
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Graham, Evarts A.	Washington Univ.
Graham, Helen T.	Washington Univ.
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Griffith, John Q., Jr.	Univ. of Pennsylvania
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Halsey, John	Tulane Univ.
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Harkins, Henry N.	Univ. of Chicago
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Harrison, R. G.	Yale Univ.
Harrison, Roland W.	Univ. of Chicago
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Hastings, A. Baird	Harvard Univ.
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Haterius, H. O.	Ohio State Univ.
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Heinbecker, Peter	Washington Univ.
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Himwich, H. E.	Albany Medical Coll.
Hines, H. M.	State Univ. of Iowa
Hinman, E. H.	Wilson Dam, Ala.
Hinman, Frank	Univ. of California Med.
Hinrichs, Marie Agnes	Univ. of Chicago
Hinsey, J. C.	Cornell Medical Coll.
Hinshaw, H. C.	Mayo Clinic
Hirschfelder, Arthur	Univ. of Minnesota
Hisaw, F. L.	Harvard Univ.
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Hobmaier, Michael	Univ. of California
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Humphrey, R. R.	Univ. of Buffalo
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Hunt, Reid	Harvard Univ.
Hunter, Warren C.	Univ. of Oregon Med.
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Irwin, M. R.	Univ. of Wisconsin
Irwin, Marion	Rockefeller Inst.
Isaacs, Raphael	Univ. of Michigan
Ivy, Andrew C.	Northwestern Univ.

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Jackson, D. E.	Univ. of Cincinnati
Jackson, Henry, Jr.	Boston City Hosp.
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Jeans, Philip C.	Univ. of Iowa
Jennings, H. S.	Johns Hopkins Univ.
Jensen, Hans F.	Univ. of California
Jobling, J. W.	Columbia Univ.
Johlin, J. M.	Vanderbilt Univ. Med.
Johnson, C. A.	Univ. of Chicago
Johnson, C. C.	Univ. of Utah
Johnson, F. H.	Princeton Univ.
Johnson, T. B.	Yale Univ.
Jolliffe, Norman H.	N. Y. Univ. Medical Coll.
Jonas, Leon	Univ. of Pennsylvania
Jones, David B.	U. S. Dept. of Agriculture
Jones, Kenneth K.	Northwestern Univ.
Jones, L. R.	St. Louis Univ.
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Juhn, Mary	Univ. of Chicago
Jukes, T. H.	Univ. of California
Julianelle, Louis A.	Washington Univ.
Jung, F. T.	Northwestern Univ. Med.
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Kamm, Oliver	Parke, Davis & Co., Detroit
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Kast, Ludwig	N. Y. Post-Graduate Med.
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Katz, Gerhard	Tulane Univ.
Katz, L. N.	Michael Reese Hosp., Chicago
Katzenelbogen, S.	Johns Hopkins Univ.

Katzman, Philip A.	St. Louis Univ.
Keefer, Chester S.	Boston City Hosp.
Keeton, Robert W.	Univ. of Illinois
Keitt, G. W.	Univ. of Wisconsin
Keller, Allen D.	Univ. of Alabama Med.
Kellogg, W. H.	Univ. of California
Kemp, Hardy A.	Baylor Univ. Medical Coll.
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Kerr, William J.	Univ. of California Med.
Kessel, John F.	Univ. of S. California
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Key, John A.	Washington Univ.
Keys, Ancel	Univ. of Minnesota
Kidd, John G.	Rockefeller Inst.
Killian, J. A.	N. Y. Post-Graduate Med.
Kindred, J. E.	Univ. of Virginia
King, Helen D.	Wistar Inst., Philadelphia
King, Joseph T.	Univ. of Minnesota
Kinsella, Ralph A.	St. Louis Univ.
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Kleiner, I. S.	N. Y. Medical Coll.
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Knoefel, P. K.	Univ. of Louisville
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Knowlton, G. C.	State Univ. of Iowa
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Kober, Philip A.	Detroit, Mich.
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Kolmer, John A.	Univ. of Pennsylvania
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Koser, Stewart	Univ. of Chicago
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Leese, Chester E.	George Washington Univ.
Leiter, Louis	Univ. of Chicago
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Leonard, S. L.	Rutgers Univ.
Leonard, Veader	Johns Hopkins Univ.
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Levin, Isaac	N. Y. City
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Levine, Max	Iowa State Coll.
Levine, Michael	Montefiore Hosp., N. Y. City
Levine, Philip	Beth Israel Hosp., Newark, N. J.
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Levy, Milton	N. Y. Univ. Medical Coll.
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Long, C. N. H.	Yale Univ.
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Lucké, Balduin	Univ. of Pennsylvania
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Lueth, H. C.	Northwestern Univ.
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Meyer, R. K.	Univ. of Wisconsin
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Norway—Birkhaug K. E., Salvesen H. A.

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